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Local Actin-Dependent Endocytosis Is Zygotically Controlled to Initiate *Drosophila* Cellularization

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SUMMARY

In early *Drosophila* embryos, several mitotic cycles proceed with aborted cytokinesis before a modified cytokinesis, called cellularization, finally divides the syncytium into individual cells. Here, we find that scission of endocytic vesicles from the plasma membrane (PM) provides a control point to regulate the furrowing events that accompany this development. At early mitotic cycles, local furrow-associated endocytosis is controlled by cell cycle progression, whereas at cellularization, which occurs in a prolonged interphase, it is controlled by expression of the zygotic gene *nullo*. *nullo* mutations impair cortical F-actin accumulation and scission of endocytic vesicles, such that membrane tubules remain tethered to the PM and deplete structural components from the furrows, precipitating furrow regression. Thus, Nullo regulates scission to restrain endocytosis of proteins essential for furrow stabilization at the onset of cellularization. We propose that developmentally regulated endocytosis can coordinate actin/PM remodeling to directly drive furrow dynamics during morphogenesis.

INTRODUCTION

Morphogenesis is the product of a developmental program that controls complex cellular mechanisms to shape cells, tissues, and whole organisms. Endocytosis represents one cellular mechanism that is tightly controlled during morphogenesis to change the position and trafficking of morphogens, signaling receptors, polarity cues, and adhesion molecules (Dudu et al., 2004; Emery and Knoblich, 2006), and thus influences cell fate and proliferation within cell populations. However, endocytosis is equally well suited to coordinate the timely remodeling of the plasma membrane (PM) and cytoskeleton at specific sites within single cells. Endocytosis adjusts the cell surface area in both dividing and crawling cells (Boucrot and Kirchhausen, 2007; Traynor and Kay, 2007) and asymmetrically distributes proteins within the cortex of polarized cells (Marco et al., 2007; Men et al., 2008). Moreover, components of the endocytic machinery are shared with the actin cytoskeleton (Smythe and Ayscough, 2006), suggesting that endocytosis couples actin and membrane dynamics. Thus, endocytosis emerges as a possible target for developmental control that could coordinate PM and actin remodeling to directly drive cell-shape change during morphogenesis.

During early morphogenesis in *Drosophila*, embryos undergo PM and actin remodeling in the form of successive rounds of furrow ingression and regression (Schejter and Wieschaus, 1993). After fertilization, the first 13 cycles of mitosis proceed with no intervening cytokinesis. Instead, at mitotic cycles 1–9, nuclei divide in the embryo interior with no associated PM furrowing. At cycle 10, the nuclei move to the embryo periphery, and during interphase of cycles 10–14, cortical domes, called somatic buds, form over each nucleus. At the onset of mitosis, the margins of somatic buds ingress as short ‘‘metaphase furrows,’’ which separate cortically anchored spindles and insure the appropriate division of DNA between adjacent nuclei. By late mitosis, these transient furrows completely regress. The result after 13 rapid mitotic cycles is a syncytial embryo filled with $\sim$6000 nuclei.

Finally, at interphase of mitotic cycle 14, the embryo completes a modified cytokinesis (Schejter and Wieschaus, 1993). During this process of cellularization, the somatic bud margins ingress between each nucleus. But rather than quickly regressing like transient metaphase furrows, cellularization furrows stably ingress and ultimately form a sheet of 40 μm tall adherent epithelial cells. The developmental switch that triggers the completion of cytokinesis at cycle 14 remains unclear. Since the entire process takes place during interphase, furrow stabilization and ingression is not likely to be regulated by cell cycle-dependent signals. Instead, expression of the zygotic genome begins just prior to the onset of cellularization and appears to differentially regulate the maternal cellular machinery to build furrows that stably ingress. Specifically, expression of only a few zygotic gene products, including *nullo*, *serendipity-α*, *slam*, and *bottleneck* (Wieschaus, 1996), controls the maternal store of proteins by as yet poorly defined mechanisms so that cellularization is initiated and completed.

Furrow dynamics in *Drosophila* embryos have classically served as a model for cytoskeletal regulation, since microtubules largely direct actin rearrangements throughout the rounds of furrowing (Foe et al., 2000), and assembly of the F-actin/Myosin-2 furrow canals at the tips of cellularization furrows preface their sustained ingression (Grosshans et al., 2005; Padash Barmchi et al., 2005; Warn et al., 1980; Warn and Magrath, 1983). However, the dramatic PM furrow dynamics suggest an additional regulatory function for the membrane trafficking machinery. Furthermore, while common machinery may initiate furrowing at both cellularization and the preceding mitotic cycles, the membrane requirements for sustained furrow ingression will necessarily vary from those of furrow regression. Comparing the mechanisms that generate ingression versus regression in these embryos thus affords a unique opportunity to understand how membrane trafficking is differentially regulated to achieve specific morphogenetic events.

PM growth at cellularization increases the embryonic surface by ~20-fold, and thus intuitively establishes a role for exocytosis in this process. Exocytosed membrane inserts at specific sites along the cellularization furrow (Lecuit and Wieschaus, 2000) and is derived from both Golgi compartments (Lee et al., 2003; Sisson et al., 2000) and recycling endosomes (Pelissier et al., 2003; Riggs et al., 2003). However, whether endocytosis contributes to furrow ingression and/or regression in these embryos is an outstanding question. Mutant and dominant-negative analysis, proteomics, and drug studies have shown that endocytosis is required for the completion of cytokinesis in a variety of other cell types (Dhonukshe et al., 2006; Feng et al., 2002; Gerald et al., 2001; Niswonger and O'Halloran, 1997; Schweitzer et al., 2005; Skop et al., 2004; Thompson et al., 2002), and that membrane endocytosed from remote sites along the PM is delivered to the division plane via the endocytic pathway (Dhonukshe et al., 2006; Schweitzer et al., 2005). Thus, endocytosis may provide components to fuel PM growth at cytokinetic furrows. At cellularization furrows, the exocytosis of membrane derived from recycling endosomes similarly implies prior endocytosis. Consistent with this, ingression of cellularization furrows is arrested by disrupting either Dynamin (Pelissier et al., 2003), which catalyzes vesicle scission from the PM and recycling endosomes, or Rab5 GTPase (Pelissier et al., 2003), which regulates early endosome dynamics. Furrow ingression also fails after perturbation of the Arfofilin-2 homolog Nuclear-fallout or Rab11 GTPase, both of which regulate recycling endosome dynamics (Pelissier et al., 2003; Riggs et al., 2003). However, while endocytosis likely accompanies cellularization, it has not been directly observed, nor has its location or timing been well defined.

We approached the current study assuming that maternal machinery is developmentally controlled to convert transient furrows of the early mitotic cycles into stable, ingressing furrows at cellularization. We find that endocytosis is one target of this developmental control. Local endocytosis occurs where furrows first ingress throughout early development, but it is differentially regulated by the zygotic gene product Nullo during cellularization. We show that *nullo* mutations impair endocytic dynamics, such that furrows are unstable and regress. We propose that Nullo acts as a developmental switch at the onset of cellularization, targeting endocytosis and so directing the assembly of stable furrows that ingress to complete cellularization.

RESULTS

Local Endocytosis Is Controlled by Cell Cycle Progression during the Early Mitotic Cycles

Successive rounds of PM furrowing in the *Drosophila* embryo likely require that membrane trafficking be tightly controlled. During mitotic cycles 10–14, we observed that components of the endocytic machinery, including Dynamin, Clathrin light chain, and Amphiphysin (Amph) localized to the margins of somatic buds and tips of metaphase furrows (Figure 1B; Figure S1, see the Supplemental Data available with this article online). Strikingly, long, thin Amph projections extended into the cytoplasm from bud margins and furrow tips (Figure 1B). Amph contains a membrane-deforming Bin-Amph-Rvs domain (BAR), and as such induces the formation of membrane tubules from liposomes in vitro (Razzaq et al., 2001; Takei et al., 1999) and from the PM in vivo (Lee et al., 2002; Peter et al., 2004). During endocytosis, this activity may constrict the neck between the PM and budding vesicle to aid membrane scission (Takei et al., 1999; Yoshida et al., 2004). Thus, Amph projections here may be membrane tubules, representing an intermediate of local endocytosis from furrow regions. Analysis of en face confocal images and volumetric renderings confirmed that Amph projections are tubules (Figure 3D; Figure S2; Movie S1). Amph tubules localized exclusively at PM regions where furrows form, and their appearance correlated with progression through the cell cycle (Figure 1B). That is, a small number of Amph tubules were seen at somatic bud margins during interphase, sharply increased at the tips of incipient metaphase furrows during prophase/metaphase, and then were completely absent at regressing furrows during telophase.

To ask how Amph tubules correlate with endocytosis in living embryos, we injected fluorophore-conjugated wheat-germ agglutinin (Alexa 488-WGA) into the perivitelline space of cycle 10–13 embryos and then imaged at the embryo midsection by time-lapse confocal microscopy. Alexa 488-WGA, which is a probe for glycosylated transmembrane proteins, bound rapidly to the cell surface immediate to the injection site (Lecuit and Wieschaus, 2000). When injected at interphase, Alexa 488-WGA concentrated in surface patches at the bud margins (Figure 1C; Movie S2). These patches mark where metaphase furrows will ingress. When injected at the interphase/prophase transition, Alexa 488-WGA labeled metaphase furrows and was concentrated at their tips. During prophase/metaphase, small numbers of Alexa 488-WGA vesicles are released from these tips (Figure 1D; Movie S3). Thus, endocytosis accompanies furrow ingression and coincides with the presence of Amph tubules observed in fixed tissue (Figures 1B–1D; Movies S2 and S3). This prophase/metaphase distribution of Alexa 488-WGA was followed by a dramatic wave of increased endocytosis that swept across the embryo surface as the cortex was maximally displaced along the polar axis (Figure 1E; Movie S4). This displacement coincides with the anaphase/telophase transition, when metaphase furrows regress (Figure S3; Movies S5 and S6). Thus, a burst of fast, vigorous endocytosis accompanies metaphase furrow regression, but is not detected by Amph tubules in fixed embryos. Since Amph tubules correspond with modest Alexa 488-WGA endocytosis at furrow ingression rather than dramatic, fast endocytosis at regression, they may represent an endocytic intermediate that is more easily captured when endocytosis is ongoing, but somehow restrained. These trends were common to mitotic cycles 10–13, suggesting that endocytic dynamics are differentially controlled by cell cycle progression at times of furrow ingression versus regression in these early embryos.

Local Endocytosis Is Controlled during Cellularization

Since Amph tubules are associated with furrow ingression, but not furrow regression, during early mitotic cycles, endocytosis