Zygotically controlled F-actin establishes cortical compartments to stabilize furrows during *Drosophila* cellularization

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Summary

Cortical compartments partition proteins and membrane at the cell surface to define regions of specialized function. Here we ask how cortical compartments arise along the plasma membrane furrows that cellularize the early *Drosophila* embryo, and investigate the influence that this compartmentalization has on furrow regression. We find that the zygotic gene product Nullo aids the establishment of discrete cortical compartments, called furrow canals, which form at the tip of incipient furrows. Upon nullo loss-of-function, proteins that are normally restricted to adjacent lateral regions of the furrow, such as Neurotactin and Discs large, spread into the furrow canals. At the same time, cortical components that should concentrate in furrow canals, such as Myosin 2 (Zipper) and Anillin (Scraps), are missing from some furrows. Depletion of these cortical components from the furrow canal compartments precipitates furrow regression. Contrary to previous models, we find that furrow compartmentalization does not require cell-cell junctions that border the furrow canals. Instead, compartmentalization is disrupted by treatments that reduce levels of cortical F-actin. Because the earliest uniform phenotype detected in nullo mutants is reduced levels of F-actin at furrow canals, we propose that Nullo compartmentalizes furrows via its regulation of F-actin, thus stabilizing furrows and insuring their ingestion to complete cellularization.

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Introduction

Cortical compartments partition cell surfaces into regions of specific function. Within these compartments the composition of the plasma membrane (PM), membrane-associated proteins and the underlying cortex is distinct from that of adjacent areas. During cytokinesis in budding yeast, compartments are defined by split Septin rings that form diffusion barriers on both sides of the F-actin/Myosin 2 contractile array to ensure cell scission (Dobbelare and Barral, 2004). Alternatively, in neurons a compartment called the initial segment is established as a dense meshwork of F-actin, Ankyrin G and Spectrin that concentrates in the cortex and binds ion channels and transmembrane proteins in the PM to restrict diffusion between the axon and cell body (Dzhashiashvili et al., 2007; Nakada et al., 2003; Winckler et al., 1999). Thus, cortical compartments are generated by at least two general mechanisms: either barriers are set up at the borders of the compartment, or a dense cortical cage assemblies just under the compartment itself.

*Drosophila* cellularization is a dramatic morphogenetic event in which PM furrows ingress between each of ~6000 cortically anchored nuclei to convert the early syncytial embryo into the cellular blastoderm (Mazumdar and Mazumdar, 2002). Discrete compartments arise along cellularization furrows as they ingress, and are first revealed by the partitioning of proteins, including F-actin and Myosin 2 (Zipper) into furrow canals that form at the tips of the incipient furrows (Warn et al., 1980; Warn and Magrath, 1983). Furrow canal compartments then lead furrow ingestion to complete cellularization. It is not currently known how furrow canal compartments are established and maintained coincident with the PM growth that drives cellularization, although several possible cellular mechanisms have now been suggested. Rho-GTPase-dependent F-actin polymerization contributes to furrow assembly (Grosshans et al., 2005; Padash Barmchi et al., 2005), and scaffolding proteins, such as Spectrin (Pesacreta et al., 1989; Thomas and Williams, 1999) and Anillin (Scraps) (Field, S. et al., 2005), accumulate at furrow canals. This dense underlying cortical matrix is likely to define the particular molecular interactions, signaling events and membrane dynamics that can occur within the furrow canal. Also, at the beginning of cellularization, E-cadherin/Catenin-based cell-cell junctions, called basal junctions, assemble immediately apical to the furrow canals (Hunter and Wieschaus, 2000; Muller and Wieschaus, 1996). These basal junctions travel in register with the furrow canals as the furrows ingress, dynamically marking the boundary between the growing PM and furrow canal. Thus, basal junctions are positioned to block lateral diffusion between adjacent PM regions, and so may maintain furrow canal composition throughout cellularization (Lecuit and Wieschaus, 2000). Lastly, the exocytosis that feeds PM growth during cellularization (Albertson et al., 2005) is targeted to the lateral PM rather than to the furrow canal (Lecuit and Wieschaus, 2000), offering an obvious means to localize membrane growth and protect furrow canal components from dilution by new membrane insertion. Although these mechanisms offer possible ways to establish and maintain furrow canal compartments, it is not clear what role each plays, or how they are regulated or coordinated with membrane growth to sustain furrow ingestion.
Zygotic transcription starts just prior to cellularization, and as such is thought to provide regulators of the maternal cellular machinery that trigger specific patterning, cell cycle and morphogenetic events (Wieschaus, 1996). A strong candidate as a zygotic regulator of cellularization is the *nullo* gene. Its transcription is purely zygotic (De Renzis et al., 2007; Rose and Wieschaus, 1992), and Nullo protein levels peak at the beginning of cellularization and then steeply decline as cellularization proceeds (Postner and Wieschaus, 1994; Rose and Wieschaus, 1992), suggesting an early role in the process. Nullo initially colocalizes with F-actin and Myosin 2 in the newly assembled furrow canals and then resolves from the furrow canals into the basal junction regions (Hunter and Wieschaus, 2000; Postner and Wieschaus, 1994), putting it in position to establish and/or maintain furrow canal compartments. *nullo* loss-of-function embryos show both basal junction and furrow canal defects (Hunter and Wieschaus, 2000; Postner and Wieschaus, 1994; Wieschaus and Sweeton, 1988), as might be predicted if basal junctions are required to establish and maintain the furrow canals. Thus, in an effort to better understand the mechanisms that partition proteins into furrow canal compartments during cellularization, and to learn how such partitioning influences furrow ingression, we focused on the relationship between furrow canal compartments and furrow dynamics in *nullo* mutant embryos.

In the experiments described below, we use various lateral membrane and furrow canal markers to follow the establishment and maintenance of cortical compartments from the beginning of cellularization. Our data suggest that Nullo is required to establish furrow canal compartments and that it does so independently of its effects on the basal junctions or the cortical scaffold protein Anillin. Instead, Nullo might establish compartments via its regulation of F-actin within the furrow. Maintenance of Myosin 2 and cortical components at the furrow canal compartments stabilizes furrows and ensures their ingression. These findings refute a widely held model that cell-cell junctions are required for furrow ingestion during *Drosophila* cellularization. Rather, we propose that it is the dense matrix of cortical F-actin that sustains ingestion to complete cellularization.

**Results**

Nullo contributes to furrow compartmentalization

At mitotic cycle 14, the embryo pauses in a prolonged interphase and cortical domes called somatic buds form over each nucleus (Schejter and Wieschaus, 1993). Cellularization starts at the sites where bud margins meet and ingress as furrows (Fig. 1A). F-actin and Myosin 2 assemble into furrow canals at these incipient furrow tips, and junctional components coalesce in adjacent basal junctions. Furrow canals then lead furrow ingestion to cellularize the embryo. We followed protein dynamics at the onset of cellularization to see when cortical compartments are clearly distinguishable along the furrow length. At the beginning of cellularization, the PDZ-containing scaffold protein Discs large (Dlg) is spread along the juxtaposed bud margins and colocalizes with lateral F-actin (Fig. 1B). However, these markers separate as bud margins transition into obvious furrows. This
takes ~5-8 minutes and, in the resulting furrows of 5 μm length, Dlg is restricted to the lateral PM, whereas F-actin clearly concentrates in the furrow canal. This asymmetric distribution of Dlg and F-actin then persists through later cellularization (Lee et al., 2003). Thus, cortical components partition into discrete compartments at a furrow length of 5 μm and this distribution is maintained throughout cellularization, coinciding in space and time with the establishment and maintenance of PM compartments (Lecuit and Wieschaus, 2000). By transmission electron microscopy (TEM), furrow canal compartments are morphologically distinct from the lateral PM in furrows measuring >5 μm in length, appearing as broadenings at the furrow tip (Fig. 1C).

How compartments arise along the cellularization furrows is not clear. nullo loss-of-function (nulloX) embryos display both furrow canal and basal junction defects (Fig. 1E) (Hunter and Wieschaus, 2000), as is predicted if basal junctions establish and/or maintain furrow canal compartments. At the beginning of cellularization, zygotically expressed Nullo concentrates with F-actin and Myosin 2 in assembling furrow canals and then resolves from the furrow canals into the basal junction regions (Fig. 1D) (Hunter and Wieschaus, 2000; Postner and Wieschaus, 1994). In nulloX embryos, basal junction components are spread along the furrows rather than coalescing into distinct basal junctions (Hunter and Wieschaus, 2000; Postner and Wieschaus, 1994; Wieschaus and Sweeton, 1988). In addition, both F-actin and Myosin 2 are missing from a fraction of furrow canal compartments, such that breaks appear in the furrow canal network (Hunter and Wieschaus, 2000; Postner and Wieschaus, 1994; Wieschaus and Sweeton, 1988). We wondered whether these previously described nulloX phenotypes correlate with compromised furrow compartmentalization.

We imaged markers that are normally restricted to the lateral PM of the cellularization furrows, and found that Dlg staining spreads into the furrow canal compartments of nulloX embryos throughout cellularization. Dlg is detected in furrow canals even at the earliest stages of cellularization when furrows have reached ~5 μm (Fig. 2A), suggesting that distinct compartments might never be established in these mutants. In addition to Dlg, the transmembrane protein Neurotactin (Nrt), which adds to the growing lateral PM at mid- to late cellularization and is restricted to this location in wild-type embryos (Lecuit and Wieschaus, 2000), is seen in all furrow canals in nulloX embryos (Fig. 2B). Thus, furrow canal compartmentalization is compromised in nulloX mutants. We noted that furrow canals are abnormally flat in nulloX mutants (Fig. 2A,B), as compared with their normal tear-shaped morphology in wild-type embryos (Fig. 1C). TEM analysis revealed that the furrow canal broadenings at the tips of some nulloX furrows had collapsed (Fig. 2C), whereas others appeared abnormally flat on their basal surface with significant membrane blebbing (Fig. 2D). According to these assays, every furrow canal compartment is compromised in nulloX embryos. This is in contrast to furrow canal breaks, which only affect a fraction of nulloX furrows. Given that every furrow canal compartment contains ectopic lateral PM components and shows altered morphology in these mutants, Nullo may directly contribute to either the establishment and/or maintenance of furrow compartmentalization.

Cortical components within the furrow canal compartments ensure sustained furrow ingression

We next asked how compromised furrow canal compartments influence furrow ingression. Despite the spreading of lateral components into all furrow canal compartments in nulloX embryos, these furrows still ingress as cellularization proceeds in these mutants. But breaks appear in the F-actin/Myosin 2 furrow canal network, indicating the absence of cortical components from some
furrow canal compartments. It has been assumed that these breaks represent sites where furrows fail to ingress, but this has not been tested. To relate furrow canal breaks with furrow dynamics we imaged Myosin 2 and the PM probe Nrt in fixed *nulloX* embryos, using furrow length as a measure of cellularization progression. In both cross-sections and single-plane images collected at the level of the furrow canals (Fig. 3A,B,D), it was clear that some breaks in the furrow canal network were due to the absence of membrane furrows. In other cases, we surprisingly found that furrows persisted but had no Myosin 2 in the furrow canal compartment. We scored the frequency of each disrupted furrow type over the course of early to mid-cellularization (Fig. 3C; 800-1200 furrows scored from 4-6 embryos per data point). At early cellularization there were more cases of ‘furrows with no Myosin 2’ than of ‘furrows missing’. As cellularization approached the fast phase of furrow ingression at 10-15 μm furrow length (Lecuit and Wieschaus, 2000), this

![Fig. 3. The absence of Myosin 2 from furrow canals precipitates furrow regression in *nulloX* embryos.](https://example.com/fig3)

(A,B,D) Confocal images of PM furrows (Nrt, red) and Myosin 2 furrow canals (Myo-2, green) ingressing between adjacent nuclei in cellularizing embryos. (A) En face images from a single plane at the level of the furrow canals showing that in *nulloX* embryos, some furrows are missing (arrows) or have no Myosin 2 in the furrow canals (arrowheads). (B) Projected z-section from a *nulloX* embryo showing adjacent nuclei separated by: (1) a furrow with Myosin 2; (2) a furrow with no Myosin 2; or (3) a missing furrow. (C) The average percentage of disrupted furrows at progressive stages of cellularization in *nulloX* embryos grown at 23°C or 29°C (total disrupted furrows, blue; furrows with no Myosin 2, red; furrows missing, green). Each point represents 4-6 cellularizing embryos of the given furrow length, with ~200 interfaces scored per embryo. Error bars represent s.d. (D) Cross-sections at progressive phases of cellularization showing furrows with Myosin 2 between all nuclei in wild-type embryos. In *nulloX* embryos, some furrows are missing (arrows) or have no Myosin 2 in the furrow canals (arrowhead). (E) Kymographs showing furrow dynamics (GFP-Spider) in live cellularizing embryos (taken from supplementary material Movies 1 and 2). Three types of furrow dynamics are seen in *nulloX* embryos: (1) furrows that ingressed ~40 μm at rates comparable to those of the wild type; (2) furrows that ingressed to lengths >5 μm then regressed; and (3) furrows that ingressed to a length of only ~5 μm then regressed. Arrows follow the tip of the regressing furrows. (F) En face confocal image from a single plane at the level of the furrow canals showing PM furrows (Nrt, red) and Anillin furrow canals (green) in cellularizing *nulloX* embryos. Some furrows are missing (arrow) or have no Anillin in the furrow canals (arrowheads). Scale bars: 5 μm in A,B,D,F.
relationship was reversed. This suggests that the furrows with no Myosin 2 in the furrow canals must have ingressed for some time but then regressed because the total number of disrupted furrows did not increase. We found that the length of neighboring furrows with and without Myosin 2 in the furrow canals did not vary significantly (ratio = 0.97 ± 0.09; n = 263 furrows with no Myosin 2, from 17 embryos with furrow lengths of 5-15 μm). Thus, furrows with no Myosin 2 in the furrow canal compartment can ingress for some distance, but appear to be unstable and are likely to regress over the course of cellularization.

We confirmed that these furrow dynamics are representative of events in living nulloX mutants using embryos that express a GFP-tagged PM probe (Gilgamesh, referred to as GFP-Spider) in a nulloX background. Confocal time-lapse images were collected at the embryo mid-section to capture cross-sections of the ingressing furrows (supplementary material Movies 1 and 2). Kymographs (time versus furrow length) were then generated to follow furrow dynamics (Fig. 3E). At the onset of cellularization, short, 5 μm-long furrows formed between all nuclei. After a brief pause, a small fraction of these incipient furrows immediately regressed; these probably represent those furrows from the fixed-tissue data that are missing from the beginning of cellularization. Among the remaining furrows that continued to ingress, most reached the full length of 40 μm at rates comparable to those of the wild type. However, others regressed throughout the process, appearing to snap back towards the embryo surface; these are likely to represent those furrows from the fixed-tissue data that lack Myosin 2 and regress at intermediate stages of cellularization. Thus, furrow kinetics in living nulloX embryos are consistent with those inferred from fixed-tissue data.

Taken together, these results demonstrate two striking relationships between furrow compartmentalization and furrow ingression. First, given that the number of furrow canals with no Myosin 2 does not increase over time, Nullo must act during compartment establishment. This is supported by the expression profile of Nullo, which peaks at the beginning of cellularization (Rose and Wieschaus, 1992). Failures in compartment establishment do not necessarily prevent furrow ingression, but correlate with later furrow regression. Second, because some furrows ingress with no Myosin 2 in the furrow canal compartment, Myosin 2 is not absolutely required within a given furrow canal in order to drive ingression of that furrow (Dawes-Hoang et al., 2005; Royou et al., 2004; Thomas and Wieschaus, 2004). Rather, Myosin 2 in furrow canal compartments stabilizes the associated furrow and sustains ingression. We also saw that additional cortical scaffolds, including Anillin and Septin (Peanut), exhibit dynamics resembling Myosin 2 in nulloX embryos (Fig. 3F; data not shown), suggesting that a number of cortical components must partition into the furrow canal compartment to stabilize but not drive furrow ingression. Conversely, furrows with no detectable F-actin in the furrow canal are almost never seen in nulloX embryos (< 0.5% furrows persist with no F-actin furrow canal; n = 1831 furrows from 20 embryos with furrow lengths of 5-15 μm). Thus, it appears that when furrow canals lack sufficient cortical components, F-actin can be depleted from the compartment and this precipitates immediate furrow regression.

Basal junctions do not establish or maintain furrow canal compartments
As shown by the localization of junctional components, nulloX mutants display disrupted basal junctions along all cellularization furrows (Fig. 1E) (Hunter and Wieschaus, 2000). This defect correlates with breaks in the F-actin/Myosin 2 furrow canal network as is predicted if basal junctions establish and/or maintain furrow canal compartments. To then determine whether disruption of basal junctions in nulloX embryos can account for the compromised furrow compartmentalization or absence of cortical components from furrow canals, we eliminated basal junctions by preparing embryos that are both maternally and zygotically deficient for armadillo (arm) and scored for furrow canal defects. arm encodes the Drosophila homolog of β-catenin (Peifer and Wieschaus, 1990) and is a known component of basal junctions (Hunter and Wieschaus, 2000; Muller and Wieschaus, 1996). In single-plane confocal images of cellularizing embryos, no Arm or E-cadherin (Shotgun) was detected in arm043A01 embryos at the position where basal junctions should form, in contrast to wild-type embryos that have thick plaques of Arm and E-cadherin at the basal junctions (Fig. 4A; data not shown). Cross-sections confirmed that Arm and E-cadherin were completely absent from the lateral PM in cellularizing arm043A01 embryos (Fig. 4B,C; data not shown). TEM of these furrows showed tight apposition between adjacent PMs in wild-type embryos, as compared with gaps in arm043A01 embryos (Fig. 4D,E). Some gaps were > 50 nm, which exceeds the distance spanned by trans-cadherin-mediated adhesion (Shapiro et al., 1995). So, as reported for other arm alleles (Cox et al., 1996; Harris and Peifer, 2004; Muller and Wieschaus, 1996), cell-cell adhesion is severely compromised in arm043A01 embryos, and no detectable basal junctions form.

Previous data indicate that arm-deficient embryos can cellularize (Grevengoed et al., 2003; Cox et al., 1996), but a full analysis of furrow canal morphology has not been reported. We examined furrow canals in arm043A01 embryos, looking for phenotypes that resemble the compartmentalization defects in nulloX mutants. We confirmed that despite the loss of basal junctions, furrows formed and ingressed around all nuclei in arm043A01 embryos. Myosin 2 is maintained in a normal pattern in all furrow canals (Fig. 4F,G), and these furrow canals contracted during late cellularization when the basal surface of the cells is normally closed-off from the yolk mass (Fig. 4F). Cross-sections further revealed that arm043A01 furrow canals are discrete tear-shaped compartments (Fig. 4G), and TEM confirmed that furrow tips broaden to form distinct furrow canal structures in these embryos (Fig. 4E). The characteristic accumulation of Nrt was observed in the growing lateral PM of arm043A01 embryos, and did not enter the furrow canals despite the absence of intervening basal junctions (Fig. 4G). Lastly, F-actin levels in furrow canals of arm043A01 embryos did not differ significantly from those of wild-type embryos (Fig. 4H, I; n = 3 independent experiments), suggesting that F-actin assembly and accumulation in furrow canals does not require basal junctions. Our inability to detect furrow defects in arm043A01 mutants argues that disrupted basal junctions are not responsible for the compromised furrow compartmentalization seen in nulloX embryos.

F-actin maintains furrow canal compartments
Since basal junctions are dispensable for establishing and maintaining furrow canal compartments, Nullo activity must compartmentalize the furrow by some alternative mechanism. In mammalian neurons, a PM compartment called the initial segment is established between the cell body and proximal region of the axon (Winckler and Mellman, 1999). Within the initial segment the lateral diffusion of lipids and proteins is limited, serving to both define a specialized compartment of the cell and to restrain the exchange of PM components between the cell body and axon. This compartment is
defined by the cortical scaffold Ankyrin G, which indirectly tethers the PM to the underlying F-actin/Spectrin meshwork (Dzhashiashvili et al., 2007; Nakada et al., 2003; Winckler et al., 1999). In *nulloX* embryos, cortical scaffold proteins such as Anillin and Septin, which can similarly link F-actin to the PM (Field and Alberts, 1995; Field, C. et al., 2005), are depleted from some furrow canals. We therefore asked whether mislocalization of these scaffolds contributes to the loss of furrow canal compartments in *nulloX* mutants.

Severe maternal-effect alleles of Anillin (*anillin*<sup>HP</sup>, *anillin*</sup>PS and *anillin*</sup>PQ) reduce the rate of furrow ingression during cellularization and induce furrow regression late in the process (Field, C. et al., 2005). Furthermore, Septin fails to localize to furrow canals in these mutants (Fig. 5A) (Field, C. et al., 2005). We examined furrow compartmentalization in embryos derived from transheterozygous *anillin*-deficient mothers (*anillin*</sup>HP/PS or *anillin*</sup>PQ/PS). We found that despite the loss of Anillin and Septin, Nullo concentrates at these

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**Fig. 4.** Basal junctions do not assemble in *arm*</sup>043A01 embryos, but functional furrow canal compartments are established and maintained. (A-C) Confocal images of PM (Nrt, red) and basal junctions (Arm, green) in cellularizing embryos. (A) En face images from a single plane at the level where basal junctions should be, showing that truncated Arm fails to accumulate in basal junctions in *arm*</sup>043A01 mutants. (B) Cross-sections of a wild-type embryo showing furrows ingressing between all nuclei and Arm accumulating at basal junctions (arrowheads). (C) Cross-sections of an *arm*</sup>043A01 mutant showing furrows ingressing between all nuclei, but no Arm accumulating at basal junctions. Truncated Arm instead accumulates apically (arrows). (D,E) TEM images showing furrows ingressing in cellularizing embryos. Arrows indicate the furrow shown at higher magnification in the right-hand images, and arrowheads indicate the position where the basal junctions should be. (D) In wild-type embryos, adjacent PMs are tightly apposed. (E) In *arm*</sup>043A01 embryos, adjacent PMs gape apart. Furrow tips broaden into furrow canals despite the absence of basal junctions. (F,G) Confocal images of Myosin 2 furrow canals (Myo-2, green) in cellularizing embryos. (F) En face images from a single plane at the level of the furrow canals at progressive phases of cellularization showing that furrow canals form around all nuclei (Early) and later constrict (Late). (G) Cross-section of *arm*</sup>043A01 mutant showing tear-shaped furrow canals that lack staining for a lateral PM probe (Nrt, red). (H) Cross-section showing F-actin (phalloidin) in cellularizing embryos. Images collected at the same settings show no difference between F-actin levels for wild-type versus *arm*</sup>043A01 embryos. (I) F-actin levels quantified in furrow canals of wild-type (black) versus *arm*</sup>043A01 (red) embryos at progressive phases of cellularization, as measured by fluorescent intensity of phalloidin staining. Each point represents one embryo in which 75-100 furrow canals were analyzed. Error bars represent s.d. Scale bars: 5 μm in A-C,F-H and 2 μm in D,E.
mutant furrow canals (Fig. 5D), consistent with Nullo acting upstream of Anillin and Septin localization during cellularization. Similar to nulloX embryos, cross-sections clearly showed that all furrow canals take on a flat morphology in embryos from either anillinHP/RS or anillinQ/RS mothers (Field, C. et al., 2005; Thomas and Wieschaus, 2004). Nonetheless, compartmentalization was unperturbed, with the lateral markers Dlg and Nrt accumulating in the lateral PM but not entering the furrow canals (Fig. 5B,C). In addition, neither Myosin 2 nor F-actin levels were compromised in furrow canals of the anillin-deficient embryos at early cellularization (data not shown; Fig. 5E,F; n=3 independent experiments). Thus, furrow compartmentalization appears to be independent of these particular cortical scaffolds.

Within the initial segment of the mammalian neuron, Ankyrin-G-dependent compartmentalization develops as F-actin accumulates in the underlying cortex (Nakada et al., 2003), and F-actin perturbation increases the mobility of lipids and proteins within the initial segment while promoting exchange of components that are normally restricted to either the cell body or axon (Nakada et al., 2003; Wincleker et al., 1999). Thus, the cortical F-actin meshwork at the initial segment is crucial to establishing and maintaining this membrane compartment. We have shown that Nullo regulates cortical F-actin levels in cellularizing embryos: in nulloX embryos, F-actin levels are reduced to approximately half those in the wild type at every persisting furrow canal from the onset of cellularization (Sokac and Wieschaus, 2008). We wondered whether the reduction in F-actin levels in nulloX mutants could account for their failure to establish and/or maintain furrow canal compartments.

To determine whether reduced F-actin levels in nulloX embryos compromise furrow canal compartmentalization, we generically reduced F-actin levels with the F-actin-destabilizing drug Cytochalasin-D (Cyto-D). Wild-type embryos (OreR) were cultured mammalian cells (Schmidt and Nichols, 2004). Previous findings show that Cyto-D treatment precipitated both breaks in the Myosin 2 furrow canal network and regression of some furrows, and this phenotype was increasingly severe at higher doses of Cyto-D (Fig. 6A). These results are consistent with a role for cortical F-actin in compartmentalizing cellularization furrows. We also found that Nullo concentrates at furrow canals following Cyto-D treatment, suggesting that Nullo localizes independently of F-actin (Fig. 6H). Interestingly, we noted that the low dose of Cyto-D also disrupted basal junctions (Fig. 6F,G), as is seen in nulloX embryos (Hunter and Wieschaus, 2000). Thus, reducing F-actin levels generically with Cyto-D essentially mimics the cellularization defects observed in nulloX mutants. These data are consistent with a model whereby Nullo establishes and maintains furrow canal compartments via its regulation of F-actin.

**Discussion**

The establishment of compartments around the cortical F-actin/Myosin 2 array appears to be a conserved feature of furrowing during conventional cytokinesis, common from yeast (Dobbelaere and Barral, 2004; Takeda et al., 2004; Wachtler et al., 2003), to sea urchin and *Xenopus* embryos (Byers and Armstrong, 1986), to cultured mammalian cells (Schmidt and Nichols, 2004). Previous
analyses in cellularizing *Drosophila* embryos similarly suggested that the F-actin/Myosin 2 furrow canals are discrete compartments that form at the tips of incipient furrows and are maintained as the furrows ingress (Lecuit and Wieschaus, 2000). According to the data we present here, compartmentalization of the cellularization furrow emerges as an essential cellular mechanism to concentrate cortical components at the furrow canal and so ensure sustained furrow ingression. We identify Nullo as a developmental regulator that aids compartment establishment and maintenance. Nullo activity serves to partition proteins along the furrow from the beginning of cellularization, retaining cortical components such as Myosin 2 at the furrow canal while excluding the lateral proteins Dlg and Nrt. In this way, Nullo stabilizes furrows such that they ingress to convert the syncytial embryo into a primary epithelial sheet.

Our data show that Nullo is unlikely to compartmentalize the cellularization furrow via basal junctions or the cortical scaffold proteins Anillin or Septin, and instead supports a model whereby Nullo regulates cortical F-actin to establish and then maintain furrow canal compartments. In *nullo* mutant embryos, furrow canal breaks and regression occur at only a fraction of furrows. This is in contrast to the global expression profile of Nullo protein. Discontinuous furrow canal phenotypes have similarly been reported following alternative perturbations of cortical F-actin in cellularizing embryos. For example, Cyto-B injection does not halt furrowing, but rather induces breaks in the Myosin 2 furrow canal network (Royou et al., 2004). RhoGEF2 and diaphanous (*dia*) mutants, which also have reduced F-actin levels at furrow canals, are also missing some furrow canals (Grosshans et al., 2005; Padash Barmchi et al., 2005).

Taken together, we now suggest that reduced F-actin compromises...
furrow canal compartments at all furrows. In support of this, we find that furrow canal morphology is altered at all furrows and lateral PM components spread into every furrow canal in nulloX mutants. However, the spreading of lateral components is not sufficient to precipitate furrow regression. Instead, it is the stochastic loss of cortical components from the furrow canal that destabilizes furrows to the extreme that they may regress. In the case of nulloX embryos, we find that even in the absence of furrow canal components such as Myosin 2, as long as the furrow canal contains some F-actin, the associated furrow continues to ingress. But these furrows appear to be sensitized and perhaps the continued dilution of furrow canal actin by the spreading lateral PM components eventually precipitates their regression.

Several published results now suggest that, in addition to Nullo, the Rho1 GTPase may contribute to furrow compartmentalization. Rho1 and its activator, RhoGEF2, localize to furrow canals and the lateral furrow membrane during cellularization, suggesting that Rho1 is specifically activated there (Grosshans et al., 2005; Padash Barmchi et al., 2005). RhoGEF2 localization is independent of F-actin (Grosshans et al., 2005), and in RhoGEF2 mutants Dia fails to accumulate at the furrow canal and the embryos have reduced levels of cortical F-actin (Padash Barmchi et al., 2005; Grosshans et al., 2005). Interestingly, RhoGEF2 and dia mutants show phenotypes strikingly similar to nulloX mutants in that some furrow canals are missing (Grosshans et al., 2005; Padash Barmchi et al., 2005). This supports our assertion that cortical F-actin helps to maintain furrow canal integrity. Furthermore, following RNAi depletions of nullo in RhoGEF2 mutants, F-actin does not assemble at furrow canals, and so the two proteins may function in separate but parallel pathways (Grosshans et al., 2005). We thus favor a model whereby the combined activities of Nullo and Rho1 provide the full complement of F-actin at and around the furrow canal, which is in turn required to establish and/or maintain the furrow canal compartment.

Why would reduced cortical F-actin compromise furrow compartmentalization? One possibility is that F-actin recruits and/or retains particular cytoskeletal or scaffold proteins at the furrow canal that are required for compartment establishment and/or maintenance. In fact, in nulloX and Cyto-D-treated embryos, Myosin 2, Anillin and Septin are missing from some furrow canal compartments. This mechanism is consistent with that of the axon initial segment, where PM compartments develop by the progressive recruitment of the scaffold protein Ankyrin G that tethers cortical F-actin/Spectrin to the PM (Dzhashashvili et al., 2007; Nakada et al., 2003; Winckler et al., 1999). The resulting meshwork traps and concentrates additional proteins in the compartment, including transmembrane receptors and ion channels (Dzhashashvili et al., 2007; Nakada et al., 2003). Alternatively, F-actin levels might control membrane trafficking events that occur at the furrow canal compartment. In support of this, we see cytoplasmic Myosin 2 punctae following F-actin perturbation in cellularizing embryos (data not shown), which might represent some form of trafficking intermediate. Since cortical F-actin modulates both endocytosis (Kaksonen et al., 2006) and exocytosis (Ehre et al., 2005; Valentinj et al., 1999), changes in actin level might change the rates of membrane and/or protein uptake and delivery within PM compartments (Gheber and Edidin, 1999; Marco et al., 2007). Lastly, F-actin levels at the furrow canal might promote lipid heterogeneities at the PM in the form of lipid micro-domains or rafts, as has been reported in a spectrum of mammalian cell types and in sea urchin embryos (Holowka et al., 2000; Oliferenko et al., 1999; Seveau et al., 2001; Wu et al., 2004).
were thus chosen and scored as one of the three types levels. Embryos were binned according to their average furrow length as measured in a cross-section at the dorsal equator. Per bin (n=4-6 embryos), the average percentages of disrupted furrows and their standard deviations were calculated. Data were plotted using MATLAB and curves fitted manually. All MATLAB source code is available upon request.

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