# Regulated Expression of *nullo* Is Required for the Formation of Distinct Apical and Basal Adherens Junctions in the *Drosophila* Blastoderm

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*Abstract.* During cellularization, the *Drosophila* embryo undergoes a large-scale cytokinetic event that packages thousands of syncytial nuclei into individual cells, resulting in the de novo formation of an epithelial monolayer in the cortex of the embryo. The formation of adherens junctions is one of the many aspects of epithelial polarity that is established during cellularization: at the onset of cellularization, the *Drosophila* β-catenin homologue Armadillo (Arm) accumulates at the leading edge of the cleavage furrow, and later to the apicolateral region where the zonula adherens precursors are formed. In this paper, we show that the basal accumulation of Arm colocalizes with DE-cadherin and Dα-catenin, and corresponds to a region of tight membrane association, which we refer to as the basal junction. Although the two junctions are similar in components and function, they differ in their response to the novel cellularization protein Nullo. Nullo is present in the basal junction and is required for its formation at the onset of cellularization. In contrast, Nullo is degraded before apical junction formation, and prolonged expression of Nullo blocks the apical clustering of junctional components, leading to morphological defects in the developing embryo. These observations reveal differences in the formation of the apical and basal junctions, and offer insight into the role of Nullo in basal junction formation.

Key words: cell division • cell adhesion • epithelial cells • intercellular junctions • developmental biology

## *Introduction*

Membrane contact between adjacent epithelial cells can trigger a cascade of events leading to the formation of stable cell–cell contacts and the establishment of cell polarity (for review see Drubin and Nelson, 1996). Upon contact, E-cadherin molecules on the cell surface form punctate adhesions which then cluster to establish larger cadherin–catenin complexes. These nascent complexes form the foundation upon which the mature zonula adherens (ZA)[1] will be built. The early contacts also induce the formation of the basolateral surface, whereas the noncontacting membrane gives rise to the apical surface of the cell.

*Drosophila* cellularization offers a powerful system for the in vivo study of adherens junction formation in the context of normal development. During cellularization, the embryo packages thousands of syncytial nuclei into individual cells (Schejter and Wieschaus, 1993), leading to the de novo formation of an epithelial monolayer and the simultaneous formation of thousands of adherens junctions. The early stages of this process take place in a syncytial environment, making them accessible to drug treatments and labeling studies. In addition, genetic approaches can be used to identify components involved in establishing epithelial monolayers. Such analysis has contributed to the characterization of the *Drosophila* homologues of E-cadherin (*shotgun*) (Tepass et al., 1996), β-catenin (*armadillo*) (Peifer and Wieschaus, 1990; Peifer et al., 1993; Müller and Wieschaus, 1996; Cox et al., 1996), and numerous genes required for the establishment of the apical and basolateral membrane domains (Müller and Wieschaus, 1996; Grawe et al., 1996; Tepass, 1996; Zarnescu and Thomas, 1999; Bhat et al., 1999; Bilder and Perrimon, 2000).

Before cellularization, the syncytial nuclei align in the cortex of the embryo, where a bulge of plasma membrane, or somatic bud, is formed above each nucleus (Foe and Alberts, 1983). Adjacent somatic buds contact each other along their bases, leading to the formation of small infoldings of plasma membrane between neighboring nuclei. During the first phase of cellularization, these infoldings are transformed into stable structures (Lecuit and Wieschaus, 2000), known as furrow canals (Fullilove and Ja-

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[1]*Abbreviation used in this paper:* ZA, zonula adherens.

cobson, 1971), which define the leading edge of the cleavage furrows. The furrow canals contain high levels of actin, myosin, and other cytokinetic proteins (Warn and Robert-Nicoud, 1990; Young et al., 1991; Field and Alberts, 1995; Fares et al., 1995), and generate a contractile force that may act in conjunction with the lateral insertion of new membrane to drive cleavage furrow invagination (Warn et al., 1990; Lecuit and Wieschaus, 2000). Once the cleavage furrows have passed the base of the nuclei, the furrow canals expand laterally to generate the basal membrane, which separates the base of the cell from the underlying yolk.

The lateral membrane forms cell–cell contacts before the completion of cellularization. Previous studies have revealed that the *Drosophila* β-catenin homologue, Armadillo (Arm), has a dynamic pattern of localization during cellularization (Müller and Wieschaus, 1996). At the onset of cellularization, Arm localizes to the leading edge of the cleavage furrows, where it remains during invagination. By the end of cellularization, this early Arm accumulation sits at the basal-most region of the lateral membrane, and is degraded during early gastrulation. A second population of Arm protein arises during mid-cellularization, along the apicolateral membrane of the cleavage furrow. During late cellularization, this protein clusters apically to form the apical spot-junctions, which give rise to the ZA during gastrulation.

The localization of Arm to the ZA precursor is not the only aspect of epithelial polarity that is initiated during cellularization. In fact, cellularization is remarkable not only for its synchronized cytokinesis, but also for the degree to which this cytokinesis is coupled to the establishment of epithelial polarity. Many components of the apical and basolateral compartments accumulate in distinct domains of the nascent cleavage furrow, and then undergo stereotypical rearrangements as epithelial polarity is resolved during gastrulation (Müller and Wieschaus, 1996; Thomas and Williams, 1999; Bhat et al., 1999; Bilder and Perrimon, 2000). The shifting distribution of Arm protein appears to represent a general pattern for the localization of adherens junction components: DE-cadherin, Dα-catenin, and $\beta_H$-spectrin are also reported to localize initially to the cellularization front, and later to accumulate in the apical spot-junctions (Oda et al., 1993, 1998; Thomas and Williams, 1999).

The existence of discrete apical and basal populations of Arm in the cleavage furrow led us to examine the possibility that two distinct spot-junction complexes are required during cellularization. In this paper we provide a morphological analysis of the basal junction, which forms at the tip of the cleavage furrow. We show that the basal junction is established at the onset of cellularization, and contains coincident accumulations of Arm, DE-cadherin, and Dα-catenin. We also demonstrate that the basal junction differs from the apical spot-junction in that it requires the presence of the novel cellularization protein, Nullo (Simpson and Wieschaus, 1990; Rose and Wieschaus, 1992; Postner and Wieschaus, 1994). Nullo protein is present in the basal junction and furrow canal until mid-cellularization, when it is rapidly degraded. *nullo* loss-of-function mutations cause defects in the formation and maintenance of the basal junction, but do not affect apical junction formation. In contrast, ectopic expression of *nullo* during late cellularization prevents the apical clustering of adherens junction components. Based on these findings, we propose a model in which Nullo protein prevents clustering of cadherin–catenin complexes, an activity that is important when multiple adherens junctions are formed within a common membrane.

## Materials and Methods

### Fly Stocks

ORE-R was used as the wild-type stock. To examine the early *nullo* phenotype, we used embryos from Df(1)6F1-2/LVII9 females carrying a *nullo*-hemagglutinin (HA)-tagged transgene on the third chromosome. *nullo* mutant embryos from this line can be identified during cycle 13 by their lack of HA staining. The ArmGAL4 line containing GAL4-VP16 under control of the zygotic Armadillo promoter was the gift of J.P. Vincent (National Institute for Medical Research, MRC, London, UK). The mat67.15 stock containing the second and third chromosomal inserts of GAL4-VP16 under the control of the maternal α-tubulin promoter was the gift of D. St. Johnston (University of Cambridge, UK). The GAL4 lines were crossed to a third chromosomal insert of the UAS*nullo* construct (N39) and control crosses were performed using flies lacking a UAS insertion.

### Constructs

The *nullo*-HA construct was created by PCR amplification of a *nullo* open reading frame cassette using a primer that introduced an NheI site just upstream of the stop codon. A fragment containing three HA repeats was inserted into the NheI site and the cassette was returned to the *nullo* genomic fragment. The genomic fragment was subcloned into the Casper4 vector for transformation (Thummel and Pirotta, 1991).

UAS*nullo* was created by PCR amplification of the *nullo* open reading frame using primers containing an upstream EcoRI site and a downstream KpnI site. These sites were used to subclone the PCR product into the pUAST vector (Brand and Perrimon, 1993).

Germline transformation was carried out by standard methods (Spradling, 1986).

### Antibody Staining and Western Blots

To visualize Armadillo, Nullo, myosin, α-catenin, or neurotactin, embryos were heat-methanol fixed as described by Wieschaus and Nusslein-Volhard (1998), and stained using mouse anti-Armadillo 7A1, mouse anti-Nullo 5C3-12, rabbit anti-myosin (gift of C. Field, Harvard Medical School, Boston, MA), rat anti-Dα-cadherin CAT2 (gift of H. Oda, ERATO, Japan Science and Technology Company, Kyoto, Japan), or mouse anti-neurotactin BP106 (Hortsch et al., 1990; obtained from the Developmental Studies Hybridoma Bank) antibodies. To visualize E-cadherin, embryos were formaldehyde fixed in a phosphate buffer as described by Oda et al. (1994) and stained with rat anti-E-Cadherin DCAD2 (gift of H. Oda). Detection of single antigens was done using an appropriate secondary antibody labeled with Alexa 568 (Molecular Probes). Double stainings were done with a combination of Alexa 546– and Alexa 488–labeled secondary antibodies (Molecular Probes). To visualize actin and Nullo-HA, embryos were fixed in 18.5% formaldehyde saturated with heptane and manually devitellinized. The HA tag was detected using mouse anti-HA antibodies (Babco) and Alexa 568 anti-mouse (Molecular Probes). Actin was visualized using Alexa 488 phalloidin (Molecular Probes). All embryos were stained with Hoechst to visualize nuclei, mounted in Aquapolymount (Polysciences, Inc.) and imaged using a ZEISS LSM-510 confocal microscope.

Extracts for Western blots were made from staged, heat-methanol fixed blastoderm embryos. Nullo protein was detected using mouse anti-Nullo 5C3-12, and α-tubulin was detected using mouse anti–α-tubulin (Sigma-Aldrich). The secondary antibody was peroxidase-labeled horse anti–mouse (Vector Laboratories) and protein detection was carried out using Renaissance Chemiluminesence Reagents (NEN Life Science Products).

### Electron Microscopy

Electron microscopy was carried out as described by Wieschaus and Nusslein-Volhard (1998).

## Results

### *Cleavage Furrows Have Two Spatially Distinct Adherens Junctions during Cellularization*

During cellularization, the syncytial blastoderm is converted to a monolayer of cells that display many of the features of a polarized epithelium, including distinct apical and basolateral surfaces that are separated by a belt-like ZA (Tepass and Hartenstein, 1994). The formation of the ZA has been well documented: it begins with an accumulation of Arm and DE-cadherin to spot-like adhesive contacts in the newly formed apicolateral membrane. During late cellularization, these accumulations cluster apically to form the apical spot-junctions (Fig. 1 A), which give rise to the ZA during gastrulation (Tepass and Hartenstein, 1994; Müller and Wieschaus, 1996; Oda et al., 1998).

In addition to its apical accumulation at the ZA, Arm protein is also localized to the leading edge of the cleavage furrows. This localization is observed at the onset of cellularization and is maintained as the membrane invaginates into the interior. When cellularization is completed, this early Arm accumulation is found at the basal-most region of the lateral membrane (Fig. 1 A). We wondered whether the localization of Arm to the basal tip of the cleavage furrow might indicate a novel requirement for adherens junctions during cellularization. As in a traditional adherens junction, the Arm protein at the cellularization front colocalizes with DE-cadherin (Fig. 1, B–D) and Dα-catenin (Fig. 1, E–G), and electron micrographs show that the membranes just above the furrow canal are more closely apposed than other regions of the lateral membranes (Fig. 1 H) (Eichenberger-Glinz, 1979; Tepass and Hartenstein, 1994). Although this region lacks the electron dense plaques of a mature ZA, it is similar in nature to the cell–cell contacts seen at the apical spot-junctions; we will therefore refer to this adhesive zone as the basal junction.

To position the basal junction relative to the furrow canal, we examined the localization of Arm with respect to myosin in the cleavage furrow. In cross section, embryos initiating cellularization show spot-like accumulations of Arm at the sites of somatic bud contact. This is similar to the accumulation of Arm seen in pseudo-cleavage furrows (our unpublished observation). The spots of Arm staining in the nascent cleavage furrows are spatially distinct from the early accumulation of myosin (Fig. 2, A–C), suggesting the basal junction and furrow canal form as separate domains. We also observed many embryos that had a clear accumulation of Arm, but lacked detectable levels of myosin, suggesting that the formation of the basal junction might precede the completion of myosin localization to the cellularization front. As cleavage furrows extend, it is clear that myosin and Arm are present in nonoverlapping regions of the cellularization front (Fig. 2, D–F). During late cellularization, the furrow canals widen and it becomes apparent that the basal Arm population marks the boundary between the existing lateral membrane and the expanding basal membrane. At this stage, embryos also begin to accumulate Arm at the apical junction (Fig. 2, G–I). As the embryo initiates gastrulation, the basal accumulation of Arm, and other junctional proteins, is gradually lost, while the apical population continues to coalesce into the mature ZA.

In summary, cleavage furrows of the *Drosophila* blastoderm form two distinct adherens junctions: a transient

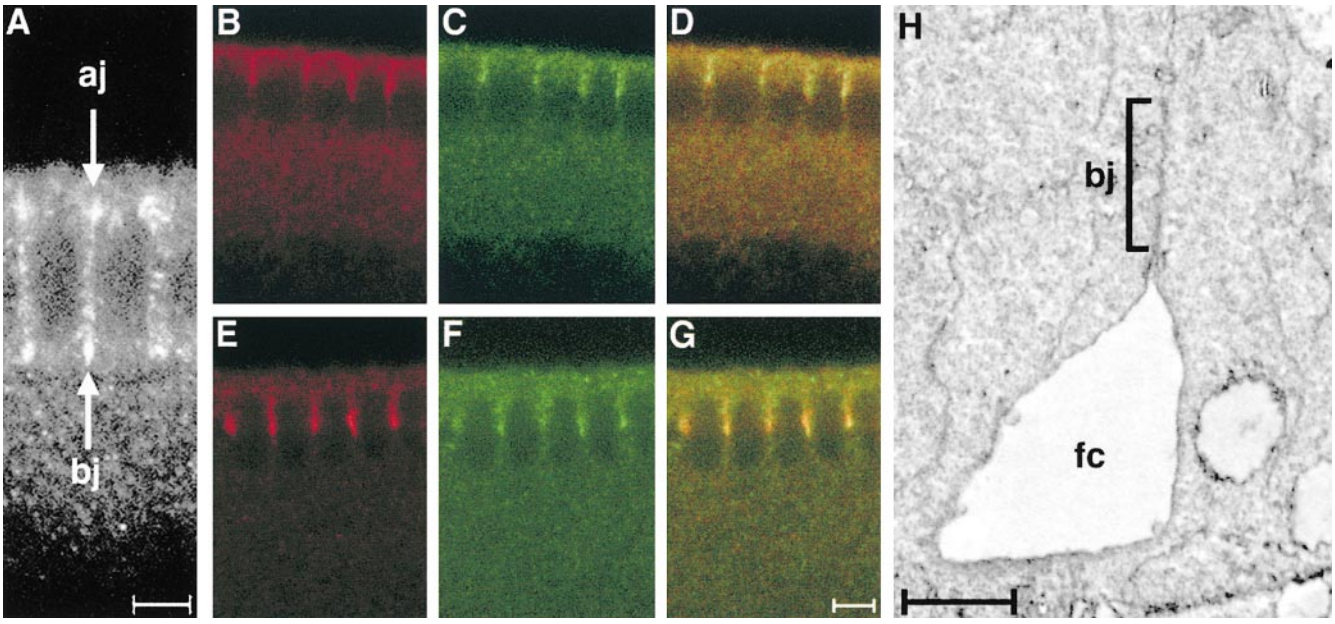


*Figure 1.* The basal junction is marked by the accumulation of Arm, DE-cadherin, and Dα-catenin. (A) A confocal cross-section of a cellularizing embryo shows that Arm accumulates to the basal junction (bj) at the leading edge of the cleavage furrow and to the apical junction (aj) at the apicolateral surface. (B–D) Arm (B) colocalizes with E-cadherin (C) at the cellularization front. (E–G) Embryos at a slightly later stage of cellularization show that Arm (E) and α-catenin (F) staining also overlap at the base of the cleavage furrow. (H) An electron micrograph showing the basal junction (bj) as a region of close membrane association, just above the furrow canal (fc) in a cellularizing embryo. Bars: (A–G) 5 μm; (H) 1 μm.

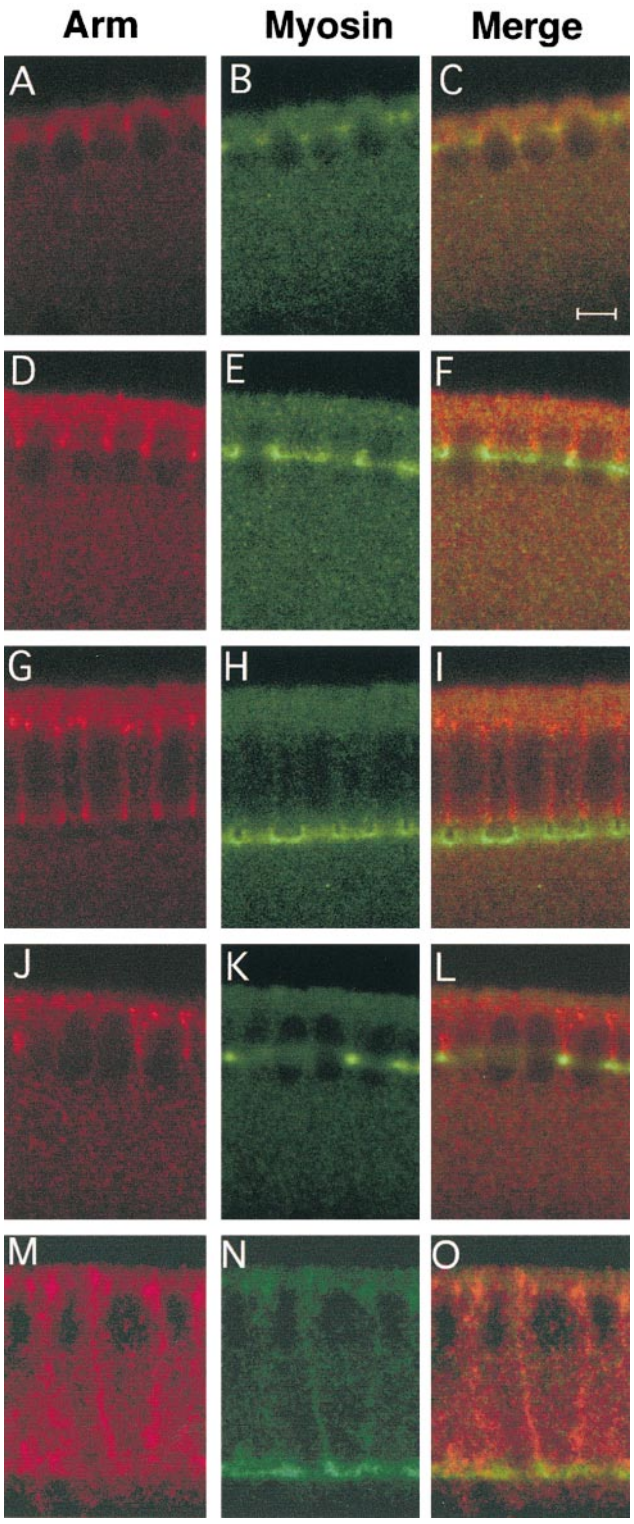


*Figure 2.* The basal junction is established as a distinct domain during early cellularization. (A–C) Before cleavage furrow invagination, Arm (red) and myosin (green) can be seen in nonoverlapping domains at the point of somatic bud contact. (D–F) As the cellularization front moves inward, Arm accumulates basally, just above the domain of myosin in the furrow canal. (G–I) In late cellularization, Arm begins to accumulate apically, while myosin remains in the expanding furrow canal. (J–L) In *nullo* mutant embryos, a subset of cleavage furrows fail to invaginate, reflected in the absence of Arm and myosin accumulation. (M–O) In cleavage furrows that do form in *nullo* mutant embryos, the basal accumulation of Arm expands towards the apical surface. Bar, 5 μm.

junction at the boundary of the basal and lateral membrane domains, and a permanent junction at the boundary of the presumptive apical and basolateral domains. Like the apical-spot junction, the basal junction contains coincident accumulations of Arm, DE-cadherin, and Dα-catenin, and corresponds to a region of tight membrane apposition.

### *The nullo Mutation Specifically Disrupts Basal Junction Formation*

The rapid rate of cellularization requires that the apical and basal junctions be formed in close spatial and temporal proximity, without coalescing to form a single junctional complex. The first indication of how this might be achieved came from our observations of Arm protein distribution in *nullo* mutant embryos. In *nullo* mutant embryos, a subset of somatic bud contacts fails to accumulate actin and myosin, and no cleavage furrows form at these positions (Simpson and Wieschaus, 1990). When we examined the distribution of myosin and Arm in the remaining furrows, we found that myosin showed its typical localization to the furrow canal (Fig. 2, J–L; also see Simpson and Wieschaus, 1990; Postner and Wieschaus, 1994), but the early Arm protein was not restricted to the basal junction. Instead, Arm extended apically along the lateral membrane (Fig. 2, M–O), suggesting that in the absence of Nullo protein, the basal Arm population moves towards the apical junction. Interestingly, the apical junction is not affected by the *nullo* mutation: during late cellularization the existing cleavage furrows form apical spot-junctions that coalesce to form ZAs. The observation that Nullo is required for basal, but not apical, junction formation is supported by the fact that Nullo protein is normally found at the basal tip of the cleavage furrow, and is degraded before apical junction formation (see below).

To pinpoint the onset of the basal junction defects, we examined the distribution of Arm protein in embryos initiating cellularization. As wild-type and *nullo* mutant embryos are phenotypically identical at this stage, we used anti–HA-Nullo immunostaining (see below) to identify the embryos. During the first phase of cellularization, cleavage furrows form at the slight infoldings of membrane where adjacent somatic buds abut each other. Surface views of wild-type embryos during this stage shows a diffuse hexagonal pattern of Arm that corresponds to the infoldings of plasma membrane (Fig. 3, A and B). The Arm staining gradually resolves into sharp lines as basal junctions form (Fig. 3, E and F), but this does not occur synchronously across the embryo. At early stages a given region contains both diffuse and sharp lines of Arm staining. This process is completed by the onset of cleavage furrow invagination, at which point all of the cleavage furrows have sharp lines of Arm accumulation at the level of the basal junction. As in apical junctions, this staining is strongest at the lateral cell–cell contacts, and Arm is depleted from the vertices of the hexagonal array.

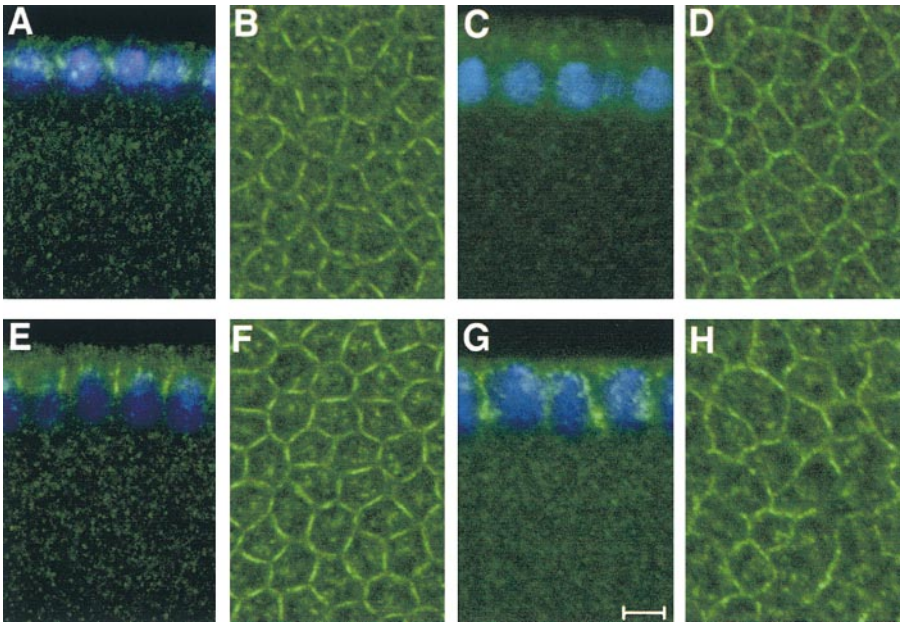


*Figure 3.* The basal junction is formed during early cellularization. (A and B) Wild-type embryos that have not begun cleavage furrow invagination show an accumulation of Arm protein (green) between nuclei (blue) at the site of somatic bud contact. A surface view at the level of Arm staining shows a hexagonal array of Arm staining, where only a subset of the interfaces have a dense accumulation of Arm, indicative of an established basal junction. (C and D) At this stage, *nullo* mutant embryos have a similar pattern of Arm accumulation at the site of somatic bud contacts. (E and F) When cleavage furrow invagination begins, Arm forms a hexagonal array where all of the interfaces now have a dense accumulation of Arm staining. (G and H) *nullo* mutant embryos have disruptions of the Arm array where cleavage furrows are absent. The remaining furrows appear to contain Arm protein, including some spots of dense accumulation, but fail to form ordered basal junctions. Bar, 5 μm.

*nullo* mutant embryos show a normal formation of somatic buds above each nucleus and the same diffuse pattern of Arm protein as wild-type embryos (Fig. 3, C and D). Arm also begins the same gradual transition to form sharp lines of staining at the level of the basal junction. However, some interfaces fail to establish a focused concentration of Arm protein, so that the partially resolved Arm network characteristic of early stages is still present when the cleavage furrows begin to invaginate (Fig. 3, G and H). Those regions that fail to form a basal junction do not give rise to cleavage furrows. Areas with a basal junction invaginate, but Arm protein does not remain restricted to the basal junction, as described above. It is interesting to note that the defects in Arm distribution can be observed before *nullo* mutant embryos develop visible morphological defects, and often can be seen before the visible accumulation of myosin to the furrow canals. This may suggest that the failure to establish a basal junction is the primary defect in *nullo* mutant embryos.

Based on these observations, we propose that the Nullo protein is required to maintain the early accumulation of Arm at the basal junction. In the absence of Nullo, a subset of somatic bud contacts contains only a diffuse accumulation of Arm protein, and fails to initiate cleavage furrows. In those furrows that do invaginate, Arm protein is not restricted to the basal junction but spreads apically along the nascent lateral membrane.

### *Nullo Protein Localizes to the Basal Junction and Furrow Canal*

The *nullo* mutation was originally thought to primarily affect the actin–myosin network that forms at the cellularization front (Simpson and Wieschaus, 1990; Rose and Wieschaus, 1992; Postner and Wieschaus, 1994). Given the apparent involvement of *nullo* in the formation of the basal junction, we were interested in examining the localization of Nullo with respect to actin, myosin, and Arm. The Nullo protein had been shown to be concentrated at the leading edge of the cleavage furrow during cellularization (Postner and Wieschaus, 1994), but the fixation conditions required to detect the protein precluded most colocalization studies. We therefore constructed a *nullo* transgene containing a triple-HA tag at the COOH terminus. The protein produced by this transgene rescues the *nullo* mutant phenotype and shows the same temporal and spatial localization as the wild-type protein, allowing us to detect Nullo under a wider range of conditions.

A comparison of Nullo and actin showed a strong colocalization during early cellularization. Both Nullo and actin are initially distributed apically, beneath the surface of the somatic buds, and then localize to the nascent cleavage furrow as it begins to invaginate (Fig. 4, A–F). Nullo and actin maintain their colocalization at the cellularization front until Nullo is degraded during late cellularization. To determine if the concentration of Nullo at the cellularization front corresponds to the basal junction, the furrow canal, or both, we examined the distribution of Nullo with respect to myosin and Arm. We found that Nullo and Arm protein distributions overlap at the basal junction (Fig. 4, G–I), whereas the region just below this contains only Nullo protein. Counterstaining with myosin confirmed that this region corresponds to a population of Nullo protein in the furrow canal (Fig. 4, J–L). Thus, the Nullo protein colocalizes not only with actin and myosin in the furrow canal, but also with actin and Arm at the basal junction.

The colocalization of Nullo with actin, Arm, and myosin

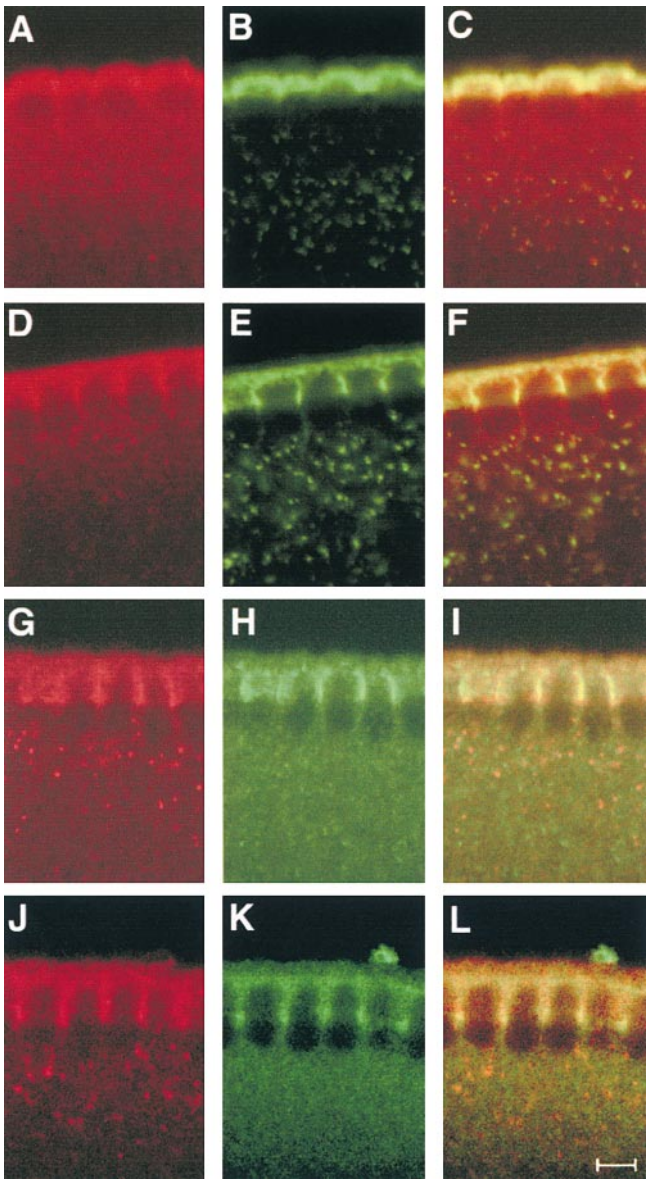


*Figure 4.* Nullo protein localization encompasses both the basal junction and the furrow canal. (A–F) Comparison of Nullo (red) and actin (green) shows that before cleavage furrow invagination, both proteins are distributed along the entire surface of the somatic buds (A–C), and gradually become concentrated to the cellularization front as the cleavage furrows begin to advance inward (D–F). (G–I) The accumulation of Nullo (red) at the cellularization front overlaps with Arm (green) in the forming basal junction. (J–L) Nullo (red) colocalizes with myosin (green) in the furrow canal. Bar, 5 μm.

is maintained until late cellularization, at which point Nullo protein is rapidly degraded. At the onset of gastrulation, the basal junction is also lost, and myosin relocalizes from the furrow canal to the apical region of the cell. This first occurs in the cells that form the ventral furrow (Fig. 5, C and E) and therefore we were curious whether Nullo protein was also degraded more rapidly in these cells. By examining embryos in cross-section, we were able to observe that the loss of Nullo protein from the cellularization front occurs more rapidly on the ventral surface of the embryo (Fig. 5, A and B).

### *Prolonged nullo Expression Disrupts Formation of the Apical Adherens Junction*

One of the most intriguing aspects of *nullo* expression is its tight temporal restriction: by late cellularization, Nullo protein has been lost from the cellularization front and *nullo* is not expressed at any later point in development (Rose and Wieschaus, 1992; Postner and Wieschaus, 1994). The abrupt degradation of Nullo protein during late cellularization suggests not only that Nullo is specifically required for cellularization, but also that it might be detrimental to later stages of development. To determine the consequences of extending Nullo expression, we placed a GAL4 responsive UAS (Brand and Perrimon, 1993) upstream of the *nullo* open reading frame and drove expression using GAL4-VP16 under the control of the maternal tubulin promoter (mat67.15). Compared with wild-type, the resulting embryos have increased levels of Nullo staining at the cellularization front (Fig. 6, A and D) and along the lateral membrane during early cellularization. During late cellularization, when the endogenous Nullo protein is degraded (Fig. 6 G), the mat67.15-UAS*nullo* embryos continue to express high levels of Nullo protein along the entire surface of the cell (Fig. 6 J). During gastrulation, the ectopic Nullo protein becomes concentrated on the lateral surface of the cells, and remains there until late embryogenesis (Fig. 6 P).

mat67.15-UAS*nullo* embryos have a normal localization of Arm to the basal junction during early cellularization (Fig. 6, A–F). However, there is a striking defect in the later localization of Arm to the apical junctions. The first differences are observed at the point when wild-type embryos lose Nullo protein and accumulate Arm along the apicolateral surface (Fig. 6, G–I). At this stage, embryos expressing UAS*nullo* maintain a normal localization of Arm at the basal junction, but fail to establish a concentrated localization of Arm in the apicolateral region (Fig. 6, J–L). Instead, low levels of Arm protein are distributed along a broad region of the lateral membrane, and fail to coalesce into a junctional structure during gastrulation (Fig. 6, M–R). Similar defects are also observed in the distribution of Dα-catenin (Fig. 5, E and F) and DE-cadherin (data not shown).

The junctional defects lead to irregularities in cell morphology and a failure to form the ventral furrow. Although the ventral cells of the mat67.15-UAS*nullo* embryos undergo a normal basal to apical shift in myosin localization and rapidly lose the basal accumulation of Arm, they are unable to invaginate. The cells do appear to initiate cell-shape changes and occasionally produce a wide, shallow furrow on the ventral surface, but they fail to complete ventral furrow formation (Fig. 4, D and F). In contrast, the cephalic furrow, which forms at the same time, appears normal (Fig. 6, M–R). As the ventral surface is normally the first region to degrade Nullo protein, it may be especially sensitive to continued Nullo expression. This may indicate that the rapid, coordinated cell constriction that forms the ventral furrow has a more stringent requirement

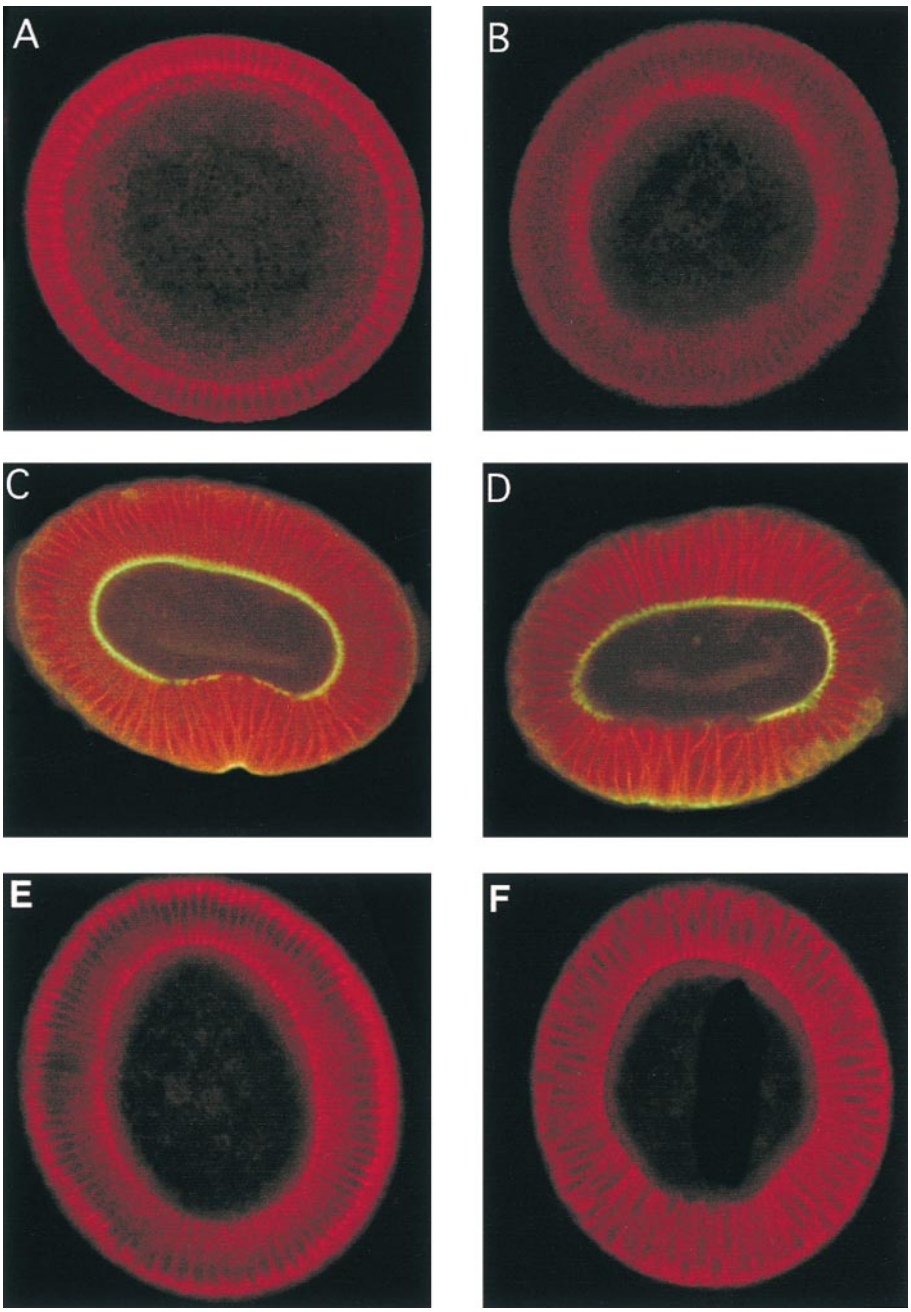


*Figure 5.* UAS*nullo*-expressing embryos have defects in ventral furrow formation. (A and B) Cross-sections of wild-type embryos just before Nullo degradation (A) and during late cellularization (B) show that loss of the Nullo protein (red) first occurs on the ventral side of the embryo. The ventral surface was identified using Twist staining (data not shown). (C and D) A wild-type (C) and UAS*nullo* embryo (D) stained with myosin (green) and counterstained with neurotactin (red) to visualize cell outlines. Although the UAS*nullo*-expressing embryo fails to complete ventral furrow formation, it has a normal redistribution of myosin to the apical surface of the ventral cells. (E and F) Staining for α-catenin (red) reveals that UAS*nullo*-expressing embryos (F) undergo a normal loss of basal α-catenin in the ventral cells, but fail to accumulate α-catenin in the apicolateral region.

for apical spot-junctions than other movements of early gastrulation.

These findings suggest that the rapid degradation of Nullo protein in late cellularization is critical for the establishment of the apical junction and the formation of the ventral furrow. Although Nullo protein is required to stabilize the accumulation of Arm in the basal junction, it appears to block the coalescence of Arm that gives rise to the apical spot-junctions.

### *Ectopic nullo Expression Does Not Affect Established Polar Epithelia*

Although mat67.15-UAS*nullo* embryos eventually form disorganized ZAs, the late embryos are highly disrupted, with cuticular holes and severe morphological defects. This made it difficult to evaluate the effect of ectopic Nullo on mature ZAs and the adherens junctions that form during later development. We therefore crossed the UAS*nullo* lines to a line carrying GAL4-VP16 under the control of the zygotic Armadillo promoter (ArmGAL4). This line initiates low levels of ectopic Nullo expression during early gastrulation (Fig. 7 A), but in later embryogenesis expresses levels comparable to the mat67.15 line (Fig. 7 B). As in the mat67.15-UAS*nullo* lines, the ectopic Nullo protein was found along the lateral surfaces of the cells.

The ArmGAL4-UAS*nullo* lines did not show any defects in adherens junctions, morphology, or cuticle formation. We compared the viability of ArmGAL4-UAS*nullo*

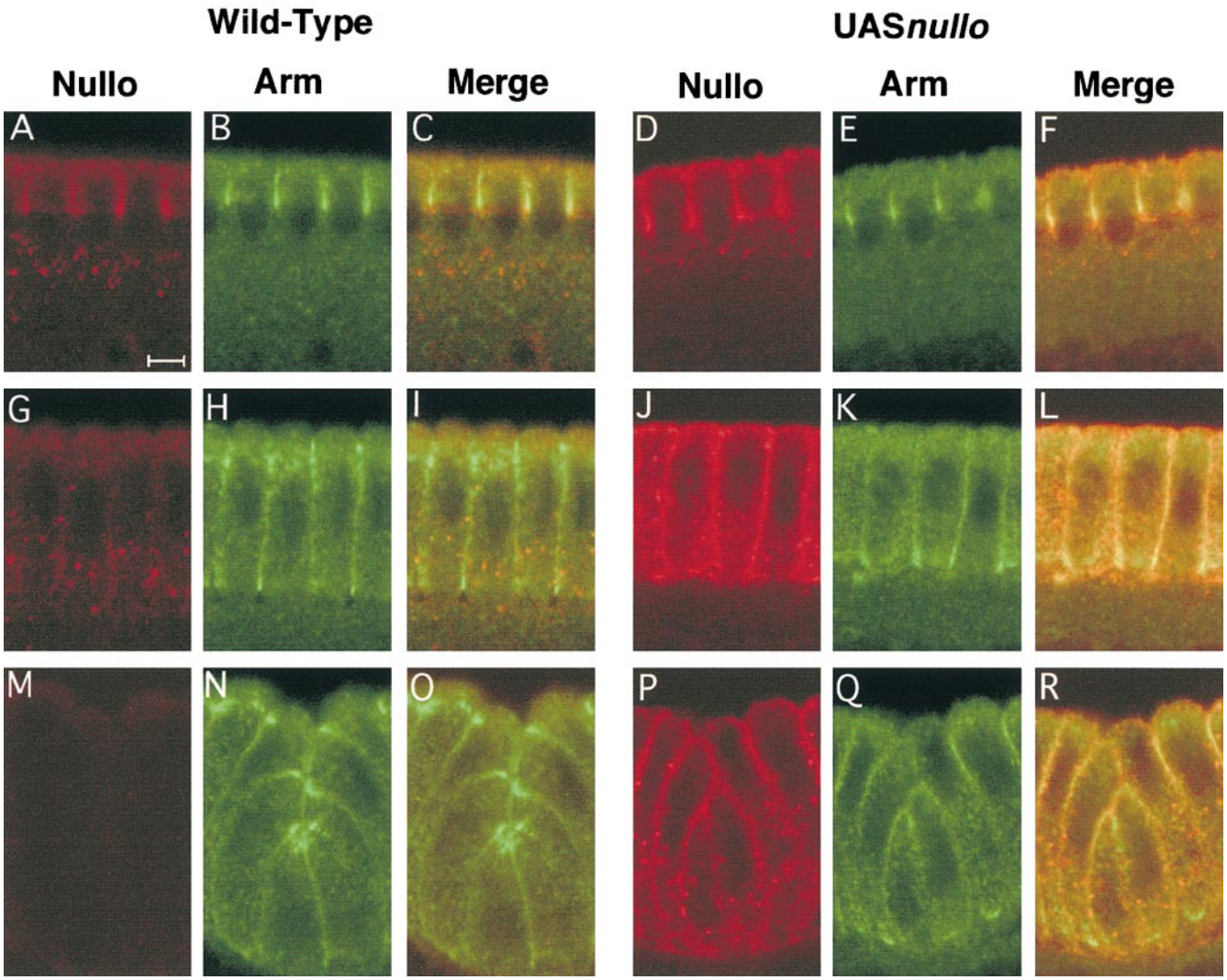


*Figure 6.* UAS*nullo*-expressing embryos fail to accumulate Arm into apical spot-junctions. (A–C) In early cellularization, wild-type embryos accumulate Nullo (red) at the cellularization front and Arm (green) in the basal junction. (D–F) At a similar stage, embryos expressing 67.15-driven UAS*nullo* have an increased level of Nullo at the cellularization front and along the apical and lateral surface of the membrane, while Arm accumulation appears normal. (G–I) By late cellularization, wild-type embryos have lost most of their Nullo expression and begin to accumulate Arm along the apicolateral surface. (J–L) At the same stage, UAS*nullo*-expressing embryos continue to have high levels of Nullo protein along the cell surface, and fail to accumulate Arm in the apicolateral region. (M–O) At the onset of gastrulation, wild-type embryos have tightly localized Arm staining at the boundary between the apical and basolateral membrane, and lack detectable levels of Nullo protein. (P–R) Gastrulating UAS*nullo*-expressing embryos have Nullo protein along the lateral surface of the cell, and still show no indication of Arm coalescence into a junctional structure. Instead, low levels of Arm protein are seen along a broad apicolateral region. Note that formation of the cephalic furrow (M–R) appears to progress normally in the absence of defined apical spot-junctions.

and mat67.15-UAS*nullo* flies to that of their balancer siblings, and found that although mat67.15-driven UAS*nullo* expression causes substantial lethality (Fig. 7 C), Arm-GAL4-driven UAS*nullo* expression has no effect on viability. This suggests that Nullo does not disrupt existing adherens junctions or the formation of new junctions in established polar epithelia. Rather, ectopic Nullo blocks only the de novo formation of apical adherens junctions that occurs as epithelial polarity is first established during cellularization.

## *Discussion*

### *Cleavage Furrows Contain Distinct Apical and Basal Junctions during Cellularization*

We have shown that the cleavage furrows of the *Drosophila* blastoderm form distinct apical and basal adherens junction complexes during cellularization. Like the apical ZA precursor, the basal junction is an area of close membrane contact that corresponds to a colocalization of Arm/β-catenin, Dα-catenin, and DE-cadherin. The basal junc-

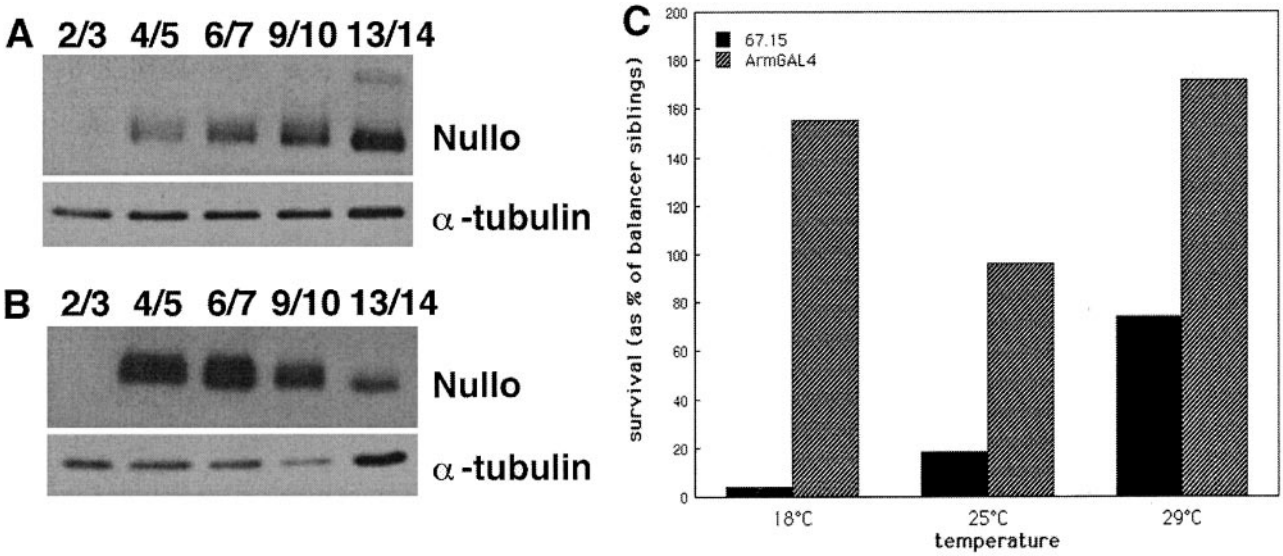


*Figure 7.* Late expression of UAS*nullo* does not affect viability. Western blots of UAS*nullo* protein expression under the control of Arm-GAL4 (A) or mat67.15 (B), with α-tubulin shown as a loading control. Stages are given according to Wieschaus and Nusslein-Volhard (1998). Expression of Nullo from the endogenous promoter is limited to cellularization (stage 4/5; Postner and Wieschaus, 1994). (C) The effect of ectopic expression on viability was assayed by crossing the GAL4 driver lines to lines carrying UAS*nullo* over a marked balancer. Survival of the UAS*nullo* progeny is given as a percentage of the survival of the balancer siblings. Arm-GAL4 driven UAS*nullo* expression does not appear to affect viability, whereas mat67.15 driven UAS*nullo* causes severe lethality, especially at 18°C. The increased lethality at lower temperatures appears to be a characteristic of the mat67.15 driver line, and not of ectopic *nullo* expression (Wieschaus, E., unpublished observations).

tion initially forms at the sites of somatic bud contact, and defines a domain that is separate from the concentration of cytokinetic proteins in the furrow canal. During midcellularization, additional Arm protein accumulates in the apicolateral region and clusters to form the ZA precursors. However, the basal junction proteins remain at the apex of the furrow canal, marking the boundary between the basal and lateral membrane compartments. The basal junction is eventually lost during gastrulation, as mature apical–basolateral polarity is established in the cells.

The formation of an adherens junction between the basal and lateral membrane compartments is unusual, and may reflect a unique need to separate these membrane domains during cellularization. The cytokinesis that takes place during cellularization is a two-step process: the lateral membrane is generated during the initial invagination of the cleavage furrow and the basal membrane is produced by the later expansion of the furrow canal (for review see Schejter and Wieschaus, 1993). Therefore, the basal membrane of the furrow canal must be isolated as the lateral cell surface elongates. The furrow canal is known to constitute a separate membrane domain (Lecuit and Wieschaus, 2000) that accumulates a specific set of proteins. It also maintains a larger intercellular space, which may prevent lateral contacts that could block furrow canal expansion. In this respect, the basal junction separates the noncontacting basal membrane from the adherent lateral membrane in a manner similar to the separation of the apical (noncontacting) and lateral (contacting) membrane by the ZA (Drubin and Nelson, 1996). The tight adhesion at the basal junction may also insulate the nascent lateral junctions from the outward pull that generates the basal cell surface (Thomas and Williams, 1999). The basal junction therefore acts to define membrane domains and reinforce cell–cell contact in a manner similar to the traditional apical adherens junction.

### *Nullo Is Required for the Formation of Multiple Junctions within a Common Membrane*

During embryonic development, spot-junctions are often created by the delivery of cadherin–catenin complexes to regions of cell–cell contact (Ohsugi et al., 1996). This appears to be the case for the basal junction: unlike Nullo and actin, which are initially present along the apical surface, Arm first appears as dots of staining at sites where somatic buds abut. The lack of overlap between Arm and myosin suggests that Arm is restricted to the small region of membrane contact between the embryo surface and the noncontacting domain of the furrow canal. This small area of localization may allow junctional components to bypass the clustering step that typically follows the delivery of the cadherin–catenin complexes. Examination of the basal Arm domain reveals that its size does not change appreciably between the onset of cleavage furrow invagination and late cellularization.

The absence of the clustering step may, in fact, be critical for the formation of the basal junctions. Unlike a typical adherens junction, which is formed at sites of cell–cell contact, the basal junction forms at sites where a single membrane folds inward and contacts itself (Fig. 8). Junctional complexes form on opposite sides of the shallow fold and establish extracellular contacts, but they are also separated by an extremely small intracellular space. In this situation, clustering is problematic: it might allow the two sides of a junction to collapse into a single complex, or al-

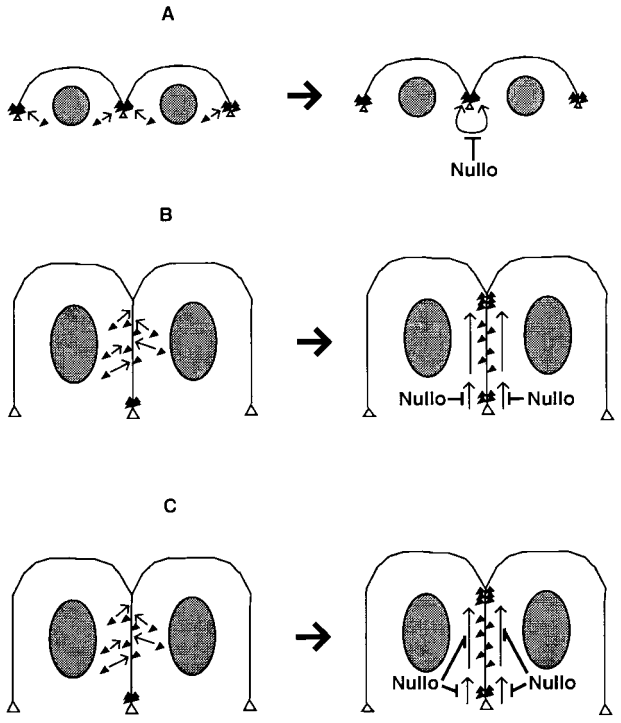


*Figure 8.* A model for the effects of Nullo on apical and basal junction formation. (A) During basal junction formation, cadherin–catenin complexes (triangles) are delivered to the small region of membrane contact between adjacent somatic buds. The presence of Nullo protein along the somatic buds blocks lateral clustering, and preserves the local accumulations of Nullo protein. (B) During apical junction formation, cadherin–catenin complexes are delivered to the broad lateral region of new membrane insertion. These complexes then undergo lateral clustering movements within the membrane to establish the apical spot-junction. The presence of Nullo at the cellularization front prevents the basal junction proteins from being recruited into the apical spot-junctions. Once the cellularization front has progressed beyond the region of apical junction formation, Nullo is no longer required to preserve the basal junction. (C) When Nullo is ectopically expressed along the lateral surface of the cells during late cellularization, it blocks the clustering which is required to form the apical junctions. This preserves the transition state, in which junctional components are distributed along a broad apicolateral zone.

low the recruitment of cadherins and catenins into neighboring furrow canals. A similar problem is faced in mid-cellularization, when the coalescence of the apical junction takes place in close proximity to the existing basal junction.

The *Drosophila* embryo must have a mechanism to preserve the local accumulations of junctional components when multiple adherens junctions are formed within a common membrane. We propose that the presence of Nullo stabilizes the accumulation of Arm in the basal junctions and prevents its recruitment into neighboring complexes or the coalescing apical junctions. We observed that in the absence of Nullo a subset of furrows fails to focus Arm protein into a stable basal junction, perhaps due to the recruitment of junctional components into neighboring complexes. The remaining basal junctions elongate during cellularization, suggesting that Arm is being recruited into the coalescing apical junctions. Nullo does not appear to be required for maintenance of the basal junction once it has moved below the region of apical junction synthesis. Although Nullo is degraded during mid-cellularization, the basal junction persists into early gastrulation, and its life is not extended in the presence of prolonged Nullo expression.

### *The Degradation of Nullo Is Critical for the Formation of the ZA*

A striking feature of *nullo* is its stringent developmental regulation: by mid-cellularization *nullo* gene expression has ceased, and the Nullo protein is rapidly degraded (Simpson and Wieschaus, 1990; Postner and Wieschaus, 1994). We have shown that extending the period of *nullo* expression into late cellularization prevents the formation of the apical adherens junctions. Instead, Arm, Dα-catenin, and DE-cadherin accumulate along a broad apicolateral region, which appears to correspond to the zone where new membrane is inserted into the cleavage furrow. This suggests that the junctional components are delivered to the lateral membrane, but fail to cluster towards the apicolateral boundary. We propose that, as in basal junction formation, the ectopic Nullo protein stabilizes the accumulation of cadherins and catenins as they are delivered to regions of lateral membrane contact. Although the depth of the contacting membrane is $<1$ μm when the basal junction is formed, it has expanded to $>20$ μm by the onset of apical junction formation. Cadherins and catenins targeted to this large area therefore must undergo conventional clustering movements to form a concentrated accumulation at the apicolateral boundary. The continued presence of Nullo protein blocks this clustering, and instead preserves the transitional state in which junctional components are broadly distributed along the lateral membrane. Ectopic expression of Nullo during late embryogenesis does not disrupt development, suggesting that once epithelial polarity is established, the presence of Nullo does not affect adherens junctions. The existence of mature apical and basolateral domains may provide cues for the targeting of cadherins and catenins, making the formation of subsequent junctions less reliant on large clustering movements, and therefore less susceptible to the effects of Nullo protein.

Cellularization is a unique process, during which two functionally distinct adherens junctions are formed de novo within a common membrane. We have shown that the precise regulation of *nullo* expression is critical for the formation of distinct basal and apical adherens junctions. Nullo protein must be present to preserve the local accumulations of cadherins and catenins needed for the formation of the basal junction. It must then be rapidly degraded to allow the extensive clustering which is required for the establishment of the apical ZA precursors. Given its contrasting effects on apical and basal junction formation, continued analysis of the Nullo protein may provide additional insights into the regulated formation of adherens junctions during development.

We thank the members of the Wieschaus and Schupbach labs for helpful discussions during these studies. We thank Joe Goodhouse for assistance with confocal microscopy, Cynthia Hsuan-Hung for maintaining stocks,

Reba Samanta for assistance with histology, and Dari Sweeton for the E.M. analysis. We thank J.P. Vincent, D. St. Johnston, C. Field, and H. Oda, and the Developmental Studies Hybridoma Bank for providing stocks and reagents. We are grateful to Thomas Lecuit, Rachel Hoang, Todd Blankenship, and Amanda Norvell for critical reading of the manuscript.

This work was supported by the Howard Hughes Medical Institute and grant 5R37HD15587 from the National Institute of Child Health and Human Development.

Submitted: 23 February 2000
Revised: 17 May 2000
Accepted: 9 June 2000

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