Molecular Biology of the Cell
Vol. 13, 146–157, January 2002

Conserved Domains of the Nullo Protein Required for Cell-Surface Localization and Formation of Adherens Junctions

Christine Hunter,* Patricia Sung,* Eyal D. Schejter,† and Eric Wieschaus*‡

*Howard Hughes Medical Institute, Department of Molecular Biology, Princeton University, Princeton, New Jersey 08540; and †Department of Molecular Genetics, Weizmann Institute of Science, Rehovot 76100, Israel

Submitted August 22, 2001; Revised October 10, 2001; Accepted October 12, 2001
Monitoring Editor: Judith Kimble

During cellularization, the *Drosophila melanogaster* embryo undergoes a transition from syncytial to cellular blastoderm with the de novo generation of a polarized epithelial sheet in the cortex of the embryo. This process couples cytokinesis with the establishment of apical, basal, and lateral membrane domains that are separated by two spatially distinct adherens-type junctions. In *nullo* mutant embryos, basal junctions fail to form at the onset of cellularization, leading to the failure of cleavage furrow invagination and the generation of multinucleate cells. Nullo is a novel protein that appears to stabilize the initial accumulation of cadherins and catenins as they form a mature basal junction. In this article we characterize a *nullo* homologue from *D. virilis* and identify conserved domains of Nullo that are required for basal junction formation. We also demonstrate that Nullo is a myristoylprotein and that the myristate group acts in conjunction with a cluster of basic amino acids to target Nullo to the plasma membrane. The membrane association of Nullo is required in vivo for its role in basal junction formation and for its ability to block apical junction formation when ectopically expressed during late cellularization.

INTRODUCTION

Drosophila cellularization is a large-scale cytokinetic event in which thousands of syncytial nuclei are simultaneously packaged into individual cells. Cleavage furrows extend from the embryonic surface between neighboring nuclei, rapidly generating a polarized epithelial sheet (reviewed in Knoblich, 2000; Schejter and Wieschaus, 1993). The apical, lateral, and basal domains of these cells are established during cleavage furrow extension by the targeted delivery of membrane components from the Golgi to the cell surface (Lecuit and Wieschaus, 2000). Domain borders are marked by adherens type junctions: the basal junction separating the basal and lateral domains during early cellularization and the apical spot junctions separating the apical and basolateral domains during late cellularization (Müller and Wieschaus, 1996; Hunter and Wieschaus, 2000). Although cellularization is a specialized process, many of the components and mechanisms of conventional cytokinesis and polarity establishment are conserved, making it a powerful system for in vivo studies of cytokinesis, cell polarity, and the establishment of cell–cell junctions.

The patterns of synchronous nuclear division and migration during the 13 division cycles preceding cellularization lead to the formation of a cortical monolayer of nuclei just beneath the embryonic surface. A bulge of plasma membrane, or somatic bud, forms above each nucleus within the cortical array (Foe and Alberts, 1983). During cellularization, regions of somatic bud contact give rise to two structures: the furrow canal and the basal junction (Lecuit and Wieschaus, 2000). The furrow canal is the basal tip of the nascent cleavage furrow and contains the presumptive basal membrane, along with cytokinetic proteins including actin, myosin, anillin, and septins (Warn and Robert-Nicoud, 1990; Young *et al.*, 1991; Fares *et al.*, 1995; Field and Alberts, 1995). Just above this structure is the basal junction, a region of tight membrane association containing the adherens junction proteins E-cadherin, α-catenin, and the β-catenin homologue Armadillo (Arm; Oda *et al.*, 1993, 1998; Hunter and Wieschaus, 2000). During the first phase of cellularization, the cleavage furrows move inward slowly as new membrane is delivered to the apical surface of the cell. Once the cleavage furrows pass the base of the nucleus, they advance rapidly as new membrane is added to the lateral cell surface (Lecuit and Wieschaus, 2000). Throughout cellularization, the furrow canal is maintained as a distinct domain, sepa-

Article published online ahead of print. Mol. Biol. Cell 10.1091/mbc.01–08-0418. Article and publication date are at www.molbiolcell.org/cgi/doi/10.1091/mbc.01–08-0418.

‡ Corresponding author. E-mail address: ewieschaus@molbio.princeton.edu.

rated from the newly generated apicolateral membrane by the basal junction. At the completion of cleavage furrow invagination, the membrane of the cleavage furrow expands laterally to form the basal surface of the cell, the basal junction is degraded, and junctional components in the lateral membrane coalesce to form permanent adherens junctions at the apical-basolateral boundary. Thus, cellularization is a complex pattern of membrane addition and junction formation that allows the coupling of cytokinesis and the establishment of cell polarity.

nullo is one of three zygotic genes specifically required for organization of the cellularization front in the *Drosophila* embryo (Merrill *et al.*, 1988; Wieschaus and Sweeton, 1988). The Nullo protein localizes to the furrow canal and basal junction during the early stages of cellularization (Postner and Wieschaus, 1994) and is required for the proper formation of the basal junction (Hunter and Wieschaus, 2000). In *nullo* mutant embryos, Arm properly accumulates at sites of somatic bud contact, but instead of forming concentrated basal junctions it expands along the lateral membrane. This leads to the failure of cleavage furrow invagination and the formation of multinucleate cells. Ectopically expressed Nullo protein can block the lateral movement of catenin–cadherin complexes that is required for coalescence of apical spot junctions. These results suggest that Nullo prevents the movement of adherens junction components within the membrane, acting to stabilize the initial accumulation of cadherins and catenins while a stable basal junction is formed.

Cloning of the *nullo* gene revealed little about potential mechanisms for its involvement in basal junction formation: *nullo* encodes a small, highly basic protein that lacks homology to any previously characterized proteins (Rose and Wieschaus, 1992). In this article we identify a homologue of Nullo from *D. virilis* and use interspecies rescue and deletion analysis to identify conserved regions of Nullo that are required for basal junction formation. We then show that Nullo is a myristoylprotein and demonstrate in vivo that N-terminal myristoylation and an N-terminal basic cluster both contribute to the membrane targeting of Nullo, although the relative contributions of these motifs changes as cellularization progresses.

MATERIALS AND METHODS

Fly Stocks

nullo mutants were obtained from the lines Df(1)6F1-2, Df(1)LVII9, Df(1)LV16 (16.3), Df(1)L-II-27–32 R5 (Rose and Wieschaus, 1992), or C(1)DX y w. Ore-R was used as the wild-type stock. The mat67.15 stock containing GAL4-VP16 under the control of the maternal α-tubulin promoter was a gift of D. St. Johnston.

Quantitation of Nullo Activity

To quantitate the severity of the *nullo* phenotype, Df(1)6F1-2 embryos were raised at 18°C, 25°C, or 29°C, and the embryos were stained using anti-Arm to visualize cell outlines and Hoechst to visualize nuclei (see below). A region containing an average of 143 nuclei was examined in 20 embryos at each temperature to determine the percentage of nuclei in multinucleate cells.

Transgenes were assayed for adult rescue activity by crossing w; P[*nullo*]/P[*nullo*] males to Df(1)6F1-2/FM7 females and scoring the number of surviving Df(1)6F1-2/Y; p[*nullo*]/+ offspring as a percentage of the Df(1)6F1-2/+; p[*nullo*]/+ offspring. These crosses were done at 18°C, 25°C, and 29°C using multiple lines carrying each transgene. Rescue at 18°C is reported relative to the background survival of Df(1)6F1-2/FM7 females crossed to males lacking a transgene.

To measure transgene rescue of the *nullo* phenotype, embryos from C(1)DX y w; P[*nullo*]/P[*nullo*] lines raised at 25°C were stained to visualize cell outlines and nuclei (see below). The percentage of embryos displaying a mutant phenotype was determined and a rescue efficiency was calculated based on the expected 25% mutant embryos produced by the C(1)DX y w stock.

To assay the activity of UAS-*nullo* deletion transgenes, males carrying the UAS transgene over a balancer were crossed to mat67.15 driver females, and the progeny were raised at 18°C. The percentage lethality is given as 1 − (surviving transgene flies/surviving balancer flies).

For transgenes that rescued the *nullo* phenotype, subcellular localization was examined in Df(1)6F1-2/Df(1)LVII9; p[*nullo*]/p[*nullo*] lines. Both Df(1)6F1-2 and Df(1)LVII9 contain additional lethal mutations, so the transheterozygote was produced to make a stock that would be viable in the presence of the *nullo* transgene. Nonrescuing transgenes were examined in nullo-X embryos produced by the C(1)DX y w; P[*nullo*]/P[*nullo*] stock.

DV nullo Cloning

A genomic *D. virilis* λ phage library (kindly provided by P. Schedl) was plated and screened with a ^{32}P-labled *D. melanogaster nullo* cDNA clone (Rose and Wieschaus, 1992), using low-stringency hybridization conditions (42°C, 29% formamide), as in O'Neil and Belote (1992). A 4.5-kb *Sac*I fragment from one of two phage clones that produced a signal through repeated rounds of hybridization was subcloned into the pBluescript (pBS; Invitrogen, Carlsbad, CA) plasmid vector and sequenced. Sequence analysis and alignments were performed using the Wisconsin Package (Genetics Computer Group, Madison, WI).

Histology

In situ hybridization to *D. virilis* embryos was done by standard methods (Tautz and Pfeifle, 1989) using a 400-base pair *Pst*I fragment from the *DV nullo* genomic clone as a probe.

To visualize Armadillo or Nullo proteins, embryos were heat-methanol fixed (Müller and Wieschaus, 1996) and stained by standard methods using mouse anti-Armadillo 7A1(Riggleman *et al.*, 1990) or mouse anti-Nullo 5C3-12 or 2F8-18 (Postner and Wieschaus, 1994). HA-tagged Nullo protein was detected using anti-HA antibodies (Covance Laboratories, Madison, WI).

To label Golgi vesicles, embryos were fixed in 18.5% formaldehyde saturated with heptane, methanol-popped, and stained using mouse anti–β-COP antibodies (gift of V. Malhotra; Ripoche *et al.*, 1994).

For actin staining, embryos were fixed in 18.5% formaldehyde saturated with heptane, manually devitellinized, and treated with Alexa 488 phalloidin.

Alexa 568–labeled antibodies (Molecular Probes, Eugene, OR) were used for single stainings, and a combination of Alexa 546– and Alexa 488–labeled secondaries (Molecular Probes) were used for double labeling. All embryos were stained with Hoechst, mounted in Aquapolymount (Polysciences, Warrington, PA), and imaged using a Zeiss LSM-510 confocal microscope (Thornwood, NY).

Deletion Constructs

Rescue assays were used to identify a 2.2-kb *Pst*I *DM nullo* genomic region that was sufficient to rescue the *nullo* phenotype. This fragment was subcloned into pBS+ (Promega, Madison, WI), and an open reading frame (ORF) cassette was created by introducing an *Nde*I site at the initiator methionine and a *Bgl*II site downstream of the stop codon using the Altered Sites Mutagenesis System (Pro-

mega). These changes did not affect the rescue activity of the *DM nullo* fragment.

The construct DMDV was produced by PCR amplification of the *DV nullo* gene using primers containing *Nde*I and *Bgl*II sites. The resulting *DV nullo* ORF cassette was then placed in the 2.2-kb *DM nullo* genomic fragment.

The ΔM mutation was created using site-directed mutagenesis to change the start of the *DM nullo* protein from MGSTHS to MASTHS. ΔE was created by amplifying a fragment of *DM nullo* corresponding to amino acids 169–213 using standard PCR. The remaining constructs were created using PCR gene SOEing (Vallejo *et al.*, 1995). ΔP to changes amino acids 35–60 of DM Nullo from KIQRLVLRKLSISARKQKRLNKRSKH to NIQNLVLNNLGVGGGGFQNGCSANTA. These changes neutralize the net charge of the unconserved N-terminal region and were based on the final 17 amino acids of the unconserved sequence from DV Nullo. ΔMP incorporates the changes from both ΔM and ΔP. ΔA removes amino acids 61–82, ΔB removes amino acids 89–102, ΔC removes amino acids 109–135, and ΔD removes amino acids 145–164.

All of the PCR products were subcloned into the *Nde*I and *Bgl*II sites of the cassette vector using *Nde*I and *Bgl*II sites on the external PCR primers. The 2.2-kb *Pst*I *nullo* genomic fragment was then subcloned into the CaSpeR-4 transformation vector (Thummel and Pirotta, 1991).

The UAS full-length *nullo* construct was created by placing a *Pst*I fragment from *nullo*-HA (Hunter and Wieschaus, 2000) into pBS KS− (Stratagene). A *Nde*I-*Sfi*I fragment was then removed from the *nullo* ORF and replaced with the corresponding fragment from each of the deletion constructs ΔM, ΔP, and ΔMP. The resulting HA-tagged deletions were PCR amplified with primers that introduced a 5′ *Eco*RI site and a 3′ *Xho*I site, and these fragments were subcloned into pUAST (Brand and Perrimon, 1993) to produce the UAS-N (full-length *nullo*), UAS-ΔM, UAS-ΔP, and UAS-ΔMP constructs.

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

Western Blots

Extracts for Western blots were made from heat-methanol–fixed blastoderm embryos. The embryo extracts were run on a 12% Tris-glycine SDS-polyacrylamide gel, and proteins were transferred onto a Hybond-P PVDF membrane (Amersham, Arlington Heights, IL). Nullo protein was detected using a 1:10 dilution of the 5C3-12 antibody. The ΔE protein was detected using the 2F8-18 antibody. α-Tubulin was detected using a 1:1000 dilution of mouse anti–α-tubulin (Sigma, St. Louis, MO). Peroxidase-labeled horse anti-mouse (Vector Laboratories, Burlingame, CA) was used as a secondary antibody, and protein detection was carried out with Renaissance Chemiluminescence Reagents (New England Nuclear, Boston, MA).

To quantitate UAS-Nullo expression, Western blot analysis was carried out as above, except that detection was performed using the ECL Plus kit (Amersham). The membrane was exposed to Hyperfilm ECL (Amersham) and then scanned and analyzed using the Storm imaging system (Molecular Dynamics, Sunnyvale, CA).

NMT Assay

nullo ORFs were mutated as described above and subcloned into the *Nde*I and *Bgl*II sites of the pRSETA (Invitrogen). BL21(DE3) *Escherichia coli* strains were cotransformed with the pRSETA construct and the pBB131 yeast N-terminal myristoyltransferase plasmid (gift of J. Gordon), and both proteins were induced with 1 mM IPTG. The N-myristoylation assay was carried out as described (Duronio *et al.*, 1990), and the final supernatants were TCA precipitated before SDS-PAGE. Samples were analyzed by Coomassie staining, Western blot, and fluorography using Amplify (Amersham).

RESULTS

Low Temperatures Compensate for the Absence of Nullo

Although Nullo is required for the rapid formation of basal junctions at the onset of cellularization, it does not appear to be an essential component of the basal junction. In wild-type embryos, Nullo protein is undetectable by late cellularization, whereas basal junctions persist until the onset of gastrulation. In addition, embryos completely lacking Nullo protein continue to form some functional basal junctions, depending on the genetic background and environmental conditions (Simpson and Wieschaus, 1990). To study factors that influence basal junction formation, we examined the *nullo* mutant line LII27.32R5, in which 20% of *nullo* hemizygous males survive to adulthood at 25°C. The R5 mutation results from a rearrangement upstream of the *nullo* ORF and does not produce sufficient Nullo protein to detect by Western blot (Rose and Wieschaus, 1992; our unpublished results). Further analysis revealed that the genomic region required for viability of the hemizygous males mapped to the same site on the X-chromosome as *nullo* (our unpublished results). This suggests that R5 does not contain a second-site suppressor, but rather produces low levels of Nullo, which are nevertheless able to influence basal junction formation.

Raising the LII27.32R5 stock at 18°C increased the proportion of viable *nullo* males. To determine if temperature would also affect the viability of *nullo* null mutations we examined a line transheterozygous for Df(1)6F1-2 and Df(1)LVII9, two small deficiencies that delete the *nullo* gene. Although this line produces no viable adults at 25°C, we could recover up to 10% of the *nullo* mutant class as viable adults at 18°C. To quantitate the effect of temperature on basal junction formation we examined Df(1) 6F1-2 embryos at 29°C, 25°C, or 18°C and determined the percentage of nuclei in multinucleate cells (Figure 1A). Wild-type embryos do not produce multinucleate cells at any of the temperatures tested. In contrast, at 29°C the majority of *nullo* mutant embryos had >80% of their nuclei in multinucleate cells (Figure 1, A and D). At 25°C, *nullo* mutant embryos had 50–80% of their nuclei in multinucleate cells. When the temperature was lowered to 18°C, there was a further reduction in the number of defective cleavage furrows: most embryos had <30% of their nuclei in multinucleate cells, and these cells were generally bi- or trinucleate (Figure 1, A and C). In addition, only 17% of the embryos had a *nullo* phenotype, as determined by anti-Arm staining of basal junctions, suggesting that the remaining 8% (of an expected 25%) of *nullo* mutant embryos were phenotypically wild type. This class may correspond to the viable mutant adults recovered at 18°C. Thus, reducing the temperature can compensate for the absence of Nullo protein and increase the number of functional basal junctions.

In wild-type embryos, Nullo is required to maintain the initial accumulation of basal junction components, allowing for the rapid construction of a stable junction before the onset of cleavage furrow invagination. This function may be more important when the nascent complexes are destabilized by high temperatures. In contrast, embryos raised at low temperatures may have an inherent stability of the

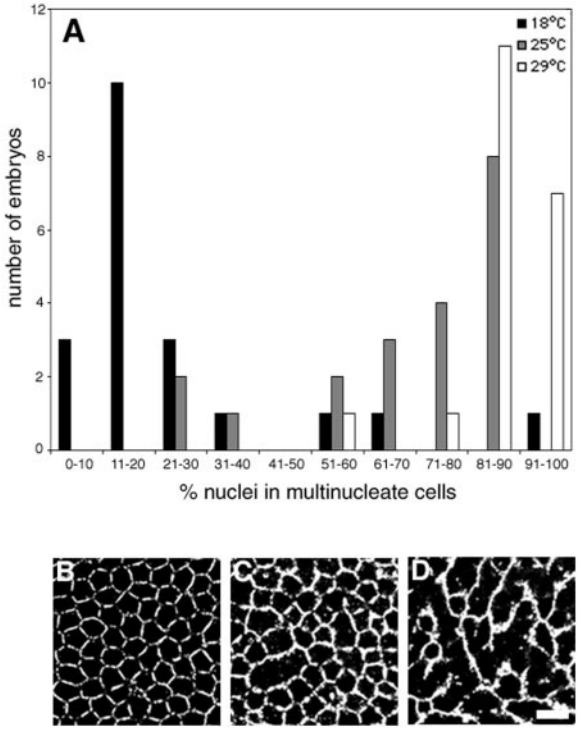

Figure 1. Decreased temperatures can partially compensate for the loss of Nullo protein. (A) Df(1) 6F1-2 embryos were raised at 18°C, 25°C, or 29°C. At each temperature, the phenotypic severity of 20 embryos was determined by counting the percentage of nuclei in multinucleate cells. The severity of the *nullo* phenotype decreased as the temperature was lowered. Surface views of a wild-type embryo raised at 18°C (B), a Df(1) 6F1-2 embryo raised at 18°C, (C) and a Df(1) 6F1-2 embryo raised at 29°C (D). Anti-Arm staining was used to visualize basal junctions. At 18°C the majority of basal junctions form in the *nullo* mutant, although a few bi- and trinucleate cells are visible. At 29°C basal junction formation is severely disrupted, with the majority of basal junctions being misformed or absent. Scale bar, 10 μm.

immature junctions, which allows them to form in the absence of Nullo.

The D. virilis nullo *Homologue*

The *nullo* gene encodes a small novel protein, with no motifs or homologies to suggest a mechanism for its involvement in basal junction formation (Rose and Wieschaus, 1992). To identify functionally important regions of the Nullo protein, we cloned a *nullo* homologue from the related species *D. virilis*. A low-stringency screen of a *D. virilis* genomic library yielded a gene (*DV nullo*) that is 55% identical to the *D. melanogaster nullo* gene within the ORF. In situ hybridization revealed that, like *D. melanogaster nullo*, the expression of *DV nullo* is restricted to a brief period at the start of cellularization (Rose and Wieschaus, 1992; Ibnsouda *et al.*, 1995). The transcript, which is absent from early embryos, accumulates rapidly throughout the embryo during the nuclear division cycles preceding cellularization and declines quickly as cellularization progresses (Figure 2, A–E). During late cellularization, the *DV nullo* transcript remains in an anterior-posterior pattern of stripes (Figure 2E), similar to that seen with *DM nullo* (Rose and Wieschaus, 1992), and it is undetectable by gastrulation (Figure 2F). The similarity between the *DM nullo* and *DV nullo* expression patterns supports the idea that the *D. virilis* gene isolated is a *nullo* homologue.

Like *DM nullo*, *DV nullo* is an intronless gene that encodes a small basic protein with a predicted size of 233 amino acids and a calculated pI of 10.8. The amino acid sequences of DM Nullo and DV Nullo are 47% identical, and the similarity of the proteins rises to 58% when conservative amino acid changes are included. Most of the conserved residues fall into clusters (Figure 2, A–E) in the C-terminal two thirds of the protein (Figure 2G). The N-terminal sequence of the proteins is comparatively unconserved, although it contains an N-terminal myristoylation site (M) and a cluster of positively charged residues (P) that are present in both proteins (Figure 2G).

We were interested in determining if the conservation between the two proteins was sufficient to allow rescue of *D. melanogaster nullo* mutant embryos using the *DV nullo* gene. We created transgenic lines that contained the *D. virilis nullo* ORF under the control of the *D. melanogaster nullo* promoter (DMDV). To assay the rescue of mild, moderate, and severe cellularization defects, we tested the ability of the transgene to restore adult viability to *nullo* mutants raised at 18°C, 25°C, or 29°C. A single copy of the *DMDV* transgene was sufficient to rescue mutant flies at all three temperatures (Figure 3A). To assess rescue of the cellularization defects directly, we examined embryos from a *nullo* mutant line that carried two copies of the *DMDV* transgene. At 25°C none of the embryos from this line had a detectable *nullo* phenotype, suggesting the transgene completely restores basal junction formation (Figure 3B).

Transgenes containing a *D. virilis* genomic fragment are also capable of rescuing *D. melanogaster nullo* mutant embryos, although the rescue activity is lower than constructs containing the *D. melanogaster* promoter region and the *D. virilis* protein coding region (our unpublished results). This would suggest that there is at least partial conservation of the regulatory regions that drive blastoderm-specific expression. These regions are likely to be contained within a small upstream region, because the *D. virilis* fragment contains only a limited region of upstream DNA. We have also observed that a *D. melanogaster nullo* transgene containing only 400 base pairs of upstream DNA is capable of reproducing the short burst of *nullo* expression, although the anterior-posterior striping seen during *nullo* degradation is less pronounced (our unpublished results). Interestingly, this small upstream region includes a regulatory motif shown to be conserved between *DM nullo* and *sry-α*, another zygotic cellularization gene with a similar pattern of expression (Ibnsouda *et al.*, 1993).

The conservation of sequence and expression pattern, along with the ability to perform interspecies rescue, confirm that the *D. virilis* gene identified is a structural and functional homologue of *D. melanogaster nullo*. The rescue experiments also identify a set of conserved features that are sufficient for Nullo activity during cellularization.

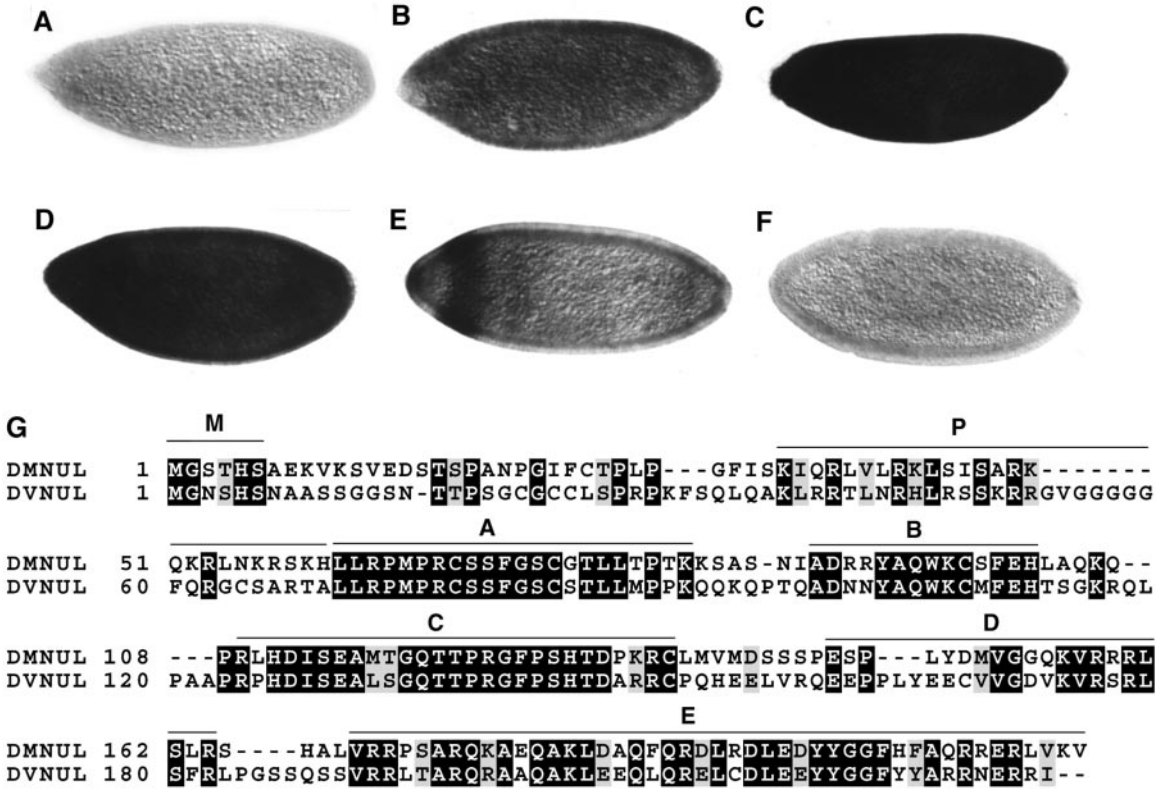

Figure 2. The *D. virilis* Nullo homologue. (A) In situ hybridization to *D. virilis* embryos shows no detectable *nullo* expression in early embryos. (B–D) Transcript levels increase just before cellularization and peak at the start of cycle 14. (E) During cellularization the transcript levels drop off abruptly in a striped pattern. (F) By the onset of gastrulation *nullo* expression is no longer detectable. This pattern is very similar to that seen for *D. melanogaster nullo* (Rose and Wieschaus, 1992). (G) A comparison of the amino acid sequences of *D. melanogaster* Nullo and *D. virilis* Nullo. Identical amino acids are shaded black, and conservative changes are indicated in gray. Both proteins contain a consensus site for N-terminal myristoylation (M) followed shortly thereafter by a positively charged cluster (P). The remainder of the protein contains five clusters of conserved amino acids (designated A–E) separated by short unconserved regions.

Identification of Conserved Regions Required for Nullo Function

The majority of the conserved amino acids are grouped into five clusters (A–E) in the C-terminal region of the Nullo protein. To assess the role of this conservation, we created a series of DM Nullo transgenes (ΔA–ΔE), each lacking a single conserved region. We then introduced these transgenes into *nullo* mutant lines, examined the expression and subcellular localization of the mutant proteins, and assayed the ability to these transgenes to rescue the *nullo* mutant phenotype.

Mutations ΔA-ΔD did not affect the level of Nullo protein expression: extracts from the transgenic embryos contained truncated proteins of the predicted size at levels comparable to wild type (Figure 4A). Surprisingly, deletion of these conserved regions had no effect on the subcellular localization of the Nullo protein. These deletion proteins accumulated at the cellularization front and showed normal punctate staining in the cortical cytoplasm (Figure 4B). ΔE deleted the epitope for the 5C3-12 anti-Nullo antibody, and although we were able to use an alternative mAb to demonstrate that this protein is produced at reduced levels, we were only able to detect faint staining in the embryo (Figure 4, A and B).

Adult rescue assays and the subsequent examination of *nullo* mutant embryos carrying two copies of each transgene revealed that ΔC and ΔD were capable of rescuing the *nullo* phenotype at 18°C, 25°C, and 29°C, whereas ΔA, ΔB, and ΔE failed to rescue at any temperature (Figure 3, A and B). The fact that ΔA and ΔB produce wild-type levels of Nullo protein, which is enriched at the cellularization front yet fail to rescue the *nullo* phenotype, suggests that these conserved regions are specifically required for Nullo to stabilize the accumulation of basal junction components in the nascent cleavage furrows.

Nullo Is Modified by N-terminal Myristoylation

Although the N-termini of the DM Nullo and DV Nullo proteins have only a limited degree of amino-acid conservation, they do contain two conserved motifs: a consensus site for N-terminal myristoylation and a cluster of positively charged amino acids. These sequences often act in concert to target proteins to the plasma membrane (reviewed in Resh,

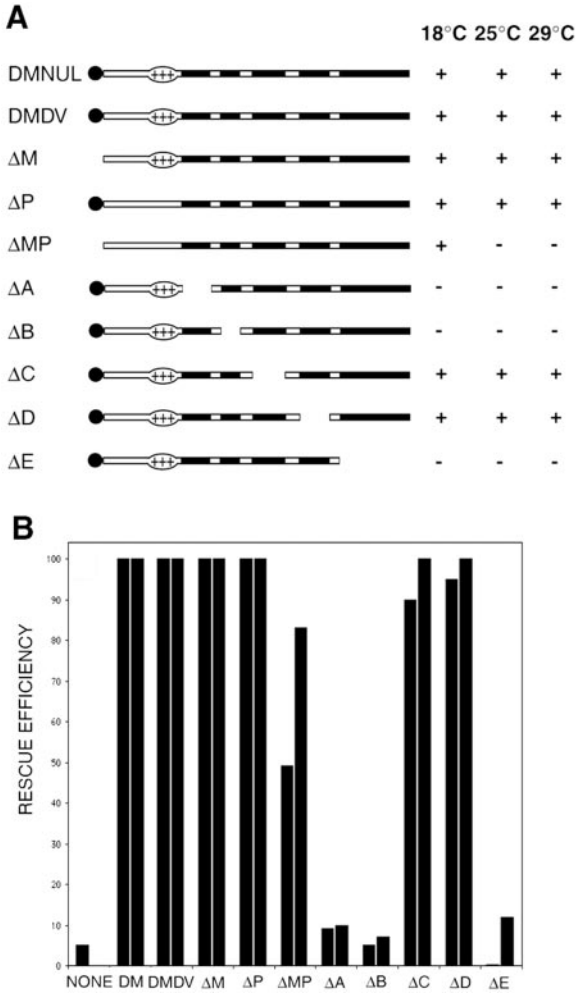

Figure 3. Rescue activity of the *nullo* transgenes. (A) The viability of Df(1)6F1-2 adult males carrying each transgene was determined at 18°C, 25°C, and 29°C. A + indicates survival above background at each temperature. (B) Embryos from C(1)DX lines homozygous for each transgene were collected at 25°C. Embryos were scored for the *nullo* phenotype, and the percentage of completely rescued mutant embryos was calculated based on the expectation that 25% of the embryos would show the *nullo* phenotype in the absence of the transgene. Data are shown for two separate insertions of each transgene.

1999), suggesting a likely role in the enrichment of Nullo at the cellularization front.

To determine if DM Nullo is a substrate for N-terminal myristoylation we expressed the *nullo* gene in a bacterial strain carrying the gene for yeast N-terminal myristoyltransferase (NMT) and grew the strains in the presence of ^{3}H-myristate (Duronio *et al.*, 1990). The resulting extracts contain Nullo protein that is labeled with ^{3}H-myristate in an NMT-dependent manner (Figure 5A). We further confirmed the specificity of this labeling by creating a glycine to alanine mutation in position two of the consensus site. This alteration has been shown in other systems to block N-myristoylation by eliminating the site of myristoyl attachment (reviewed in Resh, 1999). The Gly2 to Ala change in the Nullo protein completely blocked labeling with ^{3}H-myristate (Figure 5A).

These data indicate that the Nullo protein contains a functional N-terminal myristoylation site. Because the mechanism for N-terminal myristoylation appears to be conserved in *Drosophila* (Deichaite *et al.*, 1988; Ntwasa *et al.*, 1997; Benting *et al.*, 2000), it is likely that Nullo exists as a myristoylprotein in vivo.

N-terminal Myristoylation Is Required for Nullo Localization

To determine if the N-terminal motifs play a role in Nullo localization, we used site-directed mutagenesis to create three *DM nullo* transgenes: the first contains the Gly2 to Ala mutation that disrupts the myristoylation site (ΔM), the second has a series of point mutations that neutralize the positively charged cluster (ΔP), and the third combines these changes (ΔMP). Western blots of extracts from the transgenic embryos show that all three constructs produced protein at levels comparable to the endogenous Nullo protein (Figure 5B).

Although expressed at normal levels, each of these mutations caused specific disruptions in the subcellular localization of the Nullo protein. The wild-type Nullo protein begins to accumulate during nuclear division cycle 12 and localizes to the metaphase furrows during mitosis of cycle 13. At the start of cycle 14, Nullo protein localizes to the cell surface and then becomes enriched at the cellularization front and in punctate cytoplasmic structures (Figure 5C; Postner and Wieschaus, 1994). The identity of the punctate structures is still unknown, although they do not colocalize with actin granules or with β-COP in *cis*-Golgi–derived vesicles (our unpublished results).

Examination of ΔM revealed that, in the absence of the myristoylation site, Nullo has a normal localization to the pseudocleavage furrows during mitosis of cycle 13. However, at the onset of cellularization, Nullo adopts a nuclear localization (Figure 5C) that persists until midcellularization, when levels of localized protein decline abruptly. ΔP greatly reduces level of punctate cytoplasmic staining but has only a mild effect on membrane localization (Figure 5C). This suggests that association with the punctate cytoplasmic structures is not a prerequisite for membrane localization. The loss of both the myristoylation site and positive charge completely blocks Nullo protein localization: ΔMP is distributed diffusely throughout the cortex of the embryo (Figure 5C). These results suggest that the combination of conserved N-terminal motifs is essential for targeting Nullo protein to the cellularization front.

We then examined the ability of these mutant transgenes to rescue the *nullo* phenotype. We found that a single copy of either ΔM or ΔP was sufficient to rescue *nullo* flies to adult viability at 18°C, 25°C, or 29°C (Figure 3A) and that embryos with two copies of ΔM or ΔP have a normal hexagonal array at 25°C (Figure 3B). A single copy of ΔMP, however, partially restored viability only at 18°C. Two copies of ΔMP were capable of restoring a normal hexagonal array to 49–83% of embryos at 25°C, depending on the transgene line tested. Although ΔMP is not enriched at the plasma membrane, some portion of this protein may still reach the cellularization front. As with the hypomorph LII27.32R5, this low level of Nullo protein may increase the likelihood of basal junction formation.

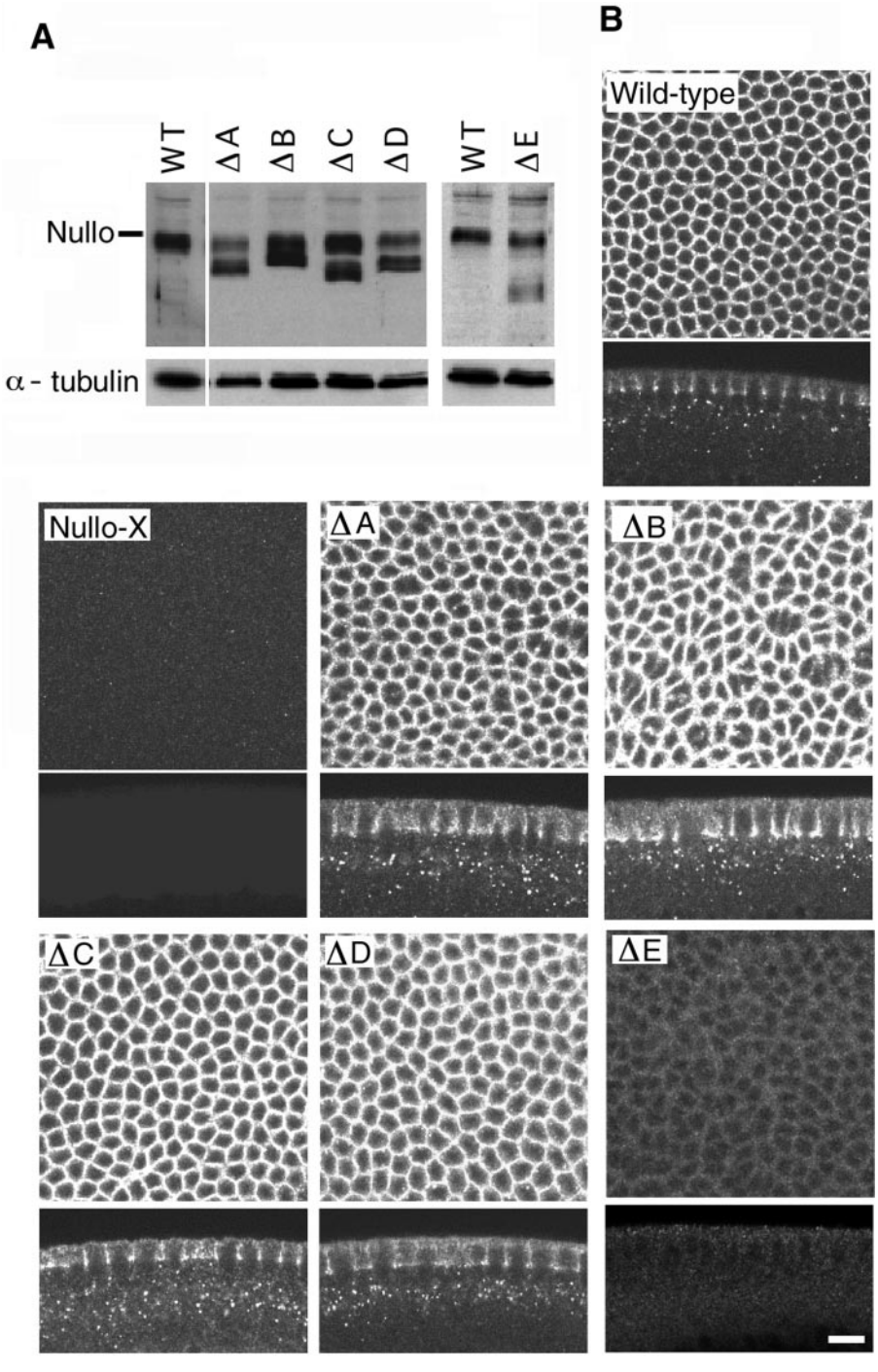

Figure 4. Conserved regions required for Nullo function. (A) Western blot showing the expression of the C-terminal deletions ΔA–ΔE. Expression of the endogenous Nullo protein is shown in the first lane. The extracts from ΔA–ΔD contain both the endogenous protein (upper band) and the deletion protein (lower band), which is present at levels equal to or greater than that of the endogenous protein. The last two lanes show extracts from wild-type and ΔE embryos in which Nullo was detected using an alternate mAb, 2F8-18. These lanes indicate that the ΔE protein is expressed at lower levels than the endogenous protein. (B) Embryos expressing only a specific deletion form of Nullo were stained with anti-Nullo antibodies to determine the subcellular localization of the mutant proteins. The top panel of each set shows a surface view, and the bottom panel shows a cross section of the cellularization front. ΔA and ΔB have a subcellular distribution similar to wild-type, but fail to rescue the *nullo* mutant phenotype. ΔC and ΔD also show a normal localization pattern and in addition are able to rescue the defects in basal junction formation. Only weak ΔE staining was detected using the 2F8-18 antibody, but there appears to be some staining above background at the level of the basal junction. Nullo-X embryos lacking a transgene are shown as a negative control. Scale bar, 10 μm.

N-terminal Motifs Are Required for the Localization of Ectopic Nullo during Late Cellularization

The wild-type Nullo protein is rapidly degraded before the completion of cellularization and the formation of the apical spot junctions (Postner and Wieschaus, 1994). When expression is artificially prolonged, Nullo prevents the clustering of cadherins and catenins into apical spot junctions, leading to defects in cell morphology and a failure of ventral furrow formation (Hunter and Wieschaus, 2000). To determine the effect of the N-terminal mutations on the localization and activity of the ectopic Nullo protein, we placed ΔM, ΔP, and ΔMP under the control of a GAL4 responsive UAS (Brand and Perrimon, 1993) and drove expression using GAL4-VP16 under the control of the maternal α-tubulin promoter.

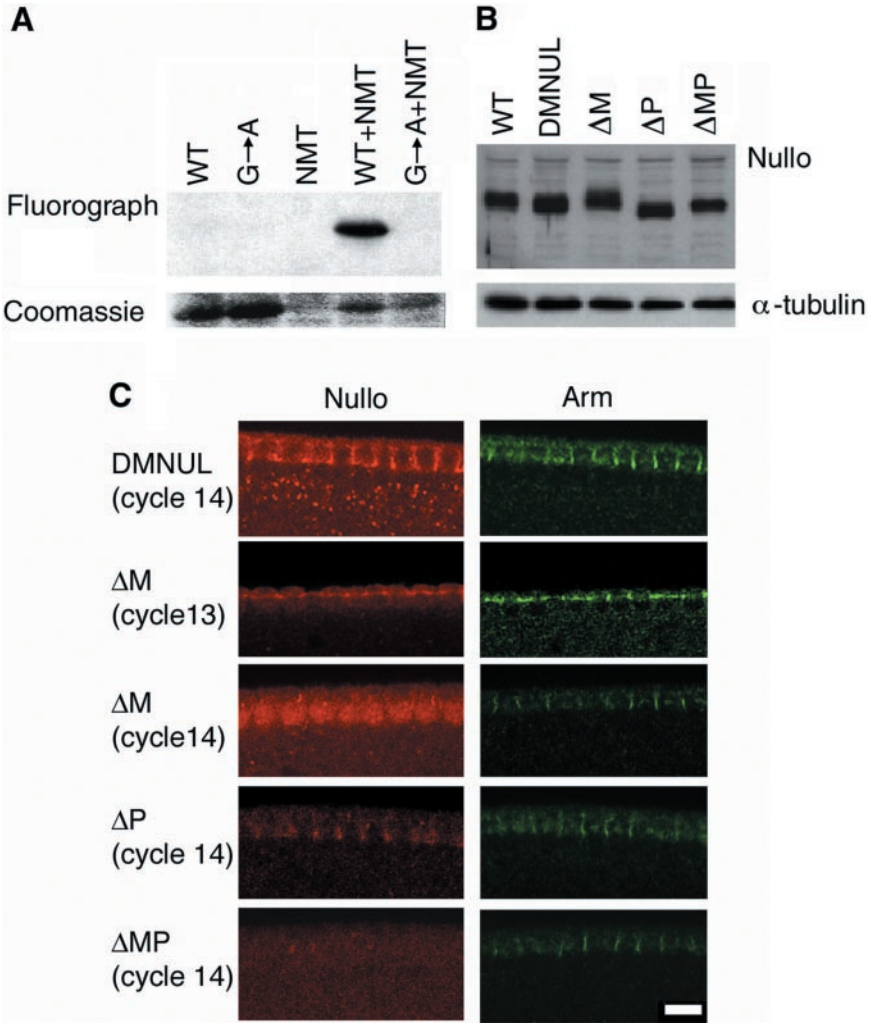

Figure 5. The N-terminal region is required for Nullo localization. (A) A fluorograph of [^{3}H]myristate–labeled extracts from bacteria expressing Nullo, Nullo containing the G2A mutation, yeast N-terminal myristoyltransferase (NMT), Nullo plus yeast NMT, or Nullo G2A plus NMT. Nullo is labeled with myristate in an NMT-dependent manner, and this labeling is blocked by the G2A mutation. Coomassie-stained Nullo protein is shown as a control. (B) Extracts from embryos expressing only the deletion form of Nullo were immunoblotted using anti-Nullo antibodies. Embryos carrying ΔM, ΔP, and ΔMP show levels of expression equivalent to that of the endogenous Nullo protein and the wild-type *nullo* transgene (DMNUL). (C) Localization of N-terminal mutants. Embryos lacking endogenous Nullo were stained with anti-Nullo antibodies and counterstained with Arm to visualize the basal junction. The full-length protein produced by the DMNUL transgene localizes to the cellularization front and shows punctate cytoplasmic staining. During cycle 13, ΔM localizes to the membrane of the pseudocleavage furrows and shows weak staining in the nucleus. During cellularization (cycle 14) ΔM relocalizes to the nucleus leaving only weak staining at the cellularization front. ΔP shows a moderate level of staining at the cellularization front but has relatively little punctate cytoplasmic staining. ΔMP has a diffuse cytoplasmic distribution in the cortex of the embryo. Scale bar, 10 μm.

This results in Nullo expression, which begins during cellularization, peaks during gastrulation, and declines gradually during late embryogenesis (Hunter and Wieschaus, 2000).

Prolonging the expression of full-length Nullo at levels characteristic of early cycle 14 is sufficient to produce the ectopic phenotype. To ensure that the potential absence of an ectopic phenotype in embryos from UAS-ΔM, UAS-ΔP, or UAS-ΔMP lines was not due to subnormal protein levels, the transgene insertions were first evaluated for protein expression levels. For each construct, several transgene insertions were analyzed by Western blot, Nullo expression levels were quantitated, and transgene insertions were assigned relative expression values. We then examined the subcellular localization of transgene insertions with similar expression levels.

When expressed during late cellularization, Nullo protein accumulates along the entire plasma membrane and in punctate structures in the basal region of the newly formed epithelial cells (Figure 6; Hunter and Wieschaus, 2000). Although ΔM under the endogenous promoter localizes to the membrane before cellularization and later becomes nuclear, UAS-ΔM expressed during late cellularization shows no membrane association and is enriched in the nucleus (Figure 6). Ectopic UAS-ΔP shows weak membrane localization, along with a diffuse cytoplasmic staining but no visible punctate staining (Figure 6), as seen with the endogenous ΔP protein. UAS-ΔMP shows no membrane staining and no observed enrichment in the nucleus versus the cytoplasm (Figure 6).

The activity of a given mutant transgene was assayed by measuring the degree of lethality caused by its ectopic expression. Using the full-length transgene to elevate Nullo protein levels during late cellularization to 69% of that observed at the beginning of cycle 14 caused lethality in 87% of embryos and blocked the formation of apical junctions (Figure 6). UAS-ΔP at similar levels caused lethality in only 43% of embryos and only resulted in a partial blockage of apical junction formation (Figure 6). UAS-ΔP therefore shows a reduction in activity but can still interfere with apical junction formation. The ectopic expression of UAS-ΔM and UAS-ΔMP, on the other hand, has no effect on viability and does not block apical junction formation (Figure 6). The failure of these mutant proteins to localize to the cell surface

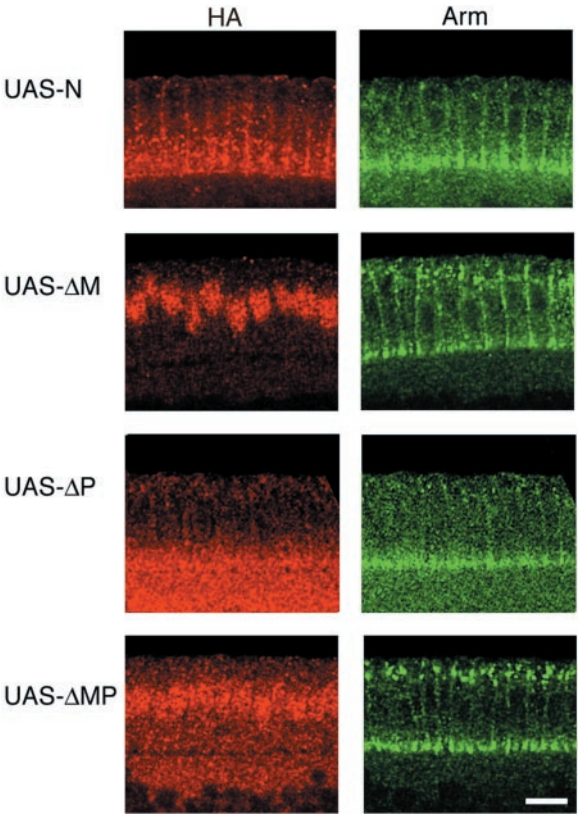

Figure 6. The N-terminal motifs are required for ectopic Nullo localization and function. UAS-N, UAS-ΔM, UAS-ΔP, and UAS-ΔMP embryos were stained with anti-HA antibody and with anti-Arm to visualize the localization of the ectopic Nullo protein and the presence or absence of apical spot junctions. The full-length UAS-N protein localizes to the membrane and the cellularization front, and shows punctate staining in the basal regions. The UAS-ΔM protein fails to localize to the membrane, but is enriched in the nucleus. The UAS-ΔP protein shows weak membrane localization, but also diffuse cytoplasmic staining and strong staining at and basal to the cellularization front. The UAS–ΔMP protein does not localize to the membrane, but shows nuclear as well as diffuse cytoplasmic staining. Although the apical spot junctions are clearly deficient in the UAS-N embryo and weak in the UAS-ΔP embryo, they appear wild-type in the UAS-ΔM and UAS-ΔMP embryos. These results suggest that membrane localization is required for blocking apical junction formation. Scale bar, 10 μm.

suggests that membrane localization is absolutely required for Nullo's interference with apical junction formation.

DISCUSSION

Comparison of D. melanogaster *and* D. virilis *Sequences Identifies Conserved Regions of Nullo*

Before the cloning of DV Nullo, little was known about the aspects of the Nullo protein involved in subcellular localization and basal junction formation. No Nullo point mutations had been isolated in screens for embryonic-lethal mutations on the X-chromosome (Nicklas and Cline, 1983; Wieschaus *et al.*, 1984; Eberl and Hilliker, 1988), and no homologues had been identified in any other species. Given the unique nature of cellularization and the fact that *nullo* expression is tightly restricted to this process, it was likely that *nullo* homologues would be found only in insects that undergo synchronous cellularization. For this reason, we turned to *D. virilis*, which undergoes cellularization but provides sufficient evolutionary divergence (~60 million years) to examine *nullo* conservation.

Like *DM nullo*, *DV nullo* appears to require a relatively small region of upstream DNA for proper expression. The DV *nullo* gene also lacks introns, a characteristic of *DM nullo* and the other zygotic cellularization genes *bottleneck* and *serendipity-α* (Vincent *et al.*, 1985; Schejter and Wieschaus, 1993). This may reflect the need to produce large amounts of transcript in the short time between the onset of zygotic transcription and cellularization. The *DV nullo* gene is expressed in the same temporal pattern as *DM nullo,* with zygotic transcription beginning just before cellularization and degradation of the transcript taking place during late cellularization. The spatial pattern is also similar, with uniform expression until midcellularization and the presence of several stripes of expression during late cellularization. As with *sry-α*, the positions of the stripes differ between *D. melanogaster* and *D. virilis* (Ibnsouda *et al.*, 1995). However, previous studies suggest that exact pattern of *nullo* expression along the anterior-poterior axis is not functionally relevant (Rose and Wieschaus, 1992).

Comparison of the DM Nullo and DV Nullo predicted proteins revealed several interesting features. The first of these is the conservation of the N-terminal myristoylation site and the presence of an adjacent unconserved, but highly basic, region. This suggested that the N-terminal myristoylation site was important for Nullo localization or function and that it likely acted as part of a "myristate plus basic" motif used in membrane localization (see below). The second feature was the presence of five distinct conserved domains, separated by short nonconserved stretches. This pattern of conservation is evident even among closely related *Drosophila* species. In the *nullo* genes of *D. oreana*, *D. lutescens*, and *D. yakuba* (Caccone *et al.*, 1996) the N-terminus and C-terminal conserved blocks have a high degree of sequence identity, whereas the C-terminal "linker" regions have already begun to diverge.

By deleting single conserved regions we identified two regions that affect Nullo activity without affecting protein localization. These proteins contain all the domains required not only to reach the plasma membrane but also to become enriched at the cellularization front. However, they still fail to rescue the basal junction phenotype, suggesting that they may define regions of Nullo that interact with components of the junction itself. Although the novel sequence of these regions makes it difficult to draw conclusions about their specific role, they provide a basis for further analysis, including the identification of interacting proteins. Two other conserved regions can be removed without affecting Nullo localization or activity, suggesting that they are dispensable or that they provide a redundant function. Truncation of the C terminus reduced protein levels, suggesting this region cannot be removed without affecting the stability of the Nullo protein. In addition to identifying functionally important regions, the deletion analysis also shed light on the previous difficulties in isolating point mutations in *nullo*.

Given the small size of the *nullo* gene, the lack of conservation in the N-terminal third of the protein and the ability to delete substantial regions of the C terminus without affecting function, the probability of isolating a lethal point-mutation in *nullo* would be quite small.

N-terminal Motifs Control the Localization of Nullo Protein

Unlike the C terminus, which has obvious regions of sequence conservation, the N-terminus of Nullo is highly divergent. Nevertheless, it contains two motifs, an N-terminal myristoylation site and a positively charged cluster, which we have shown to be essential for its localization to the cellularization front. To give rise to a stable association, the weak hydrophobic interactions between a myristate group and the plasma membrane are known to require a second contact, such as the electrostatic interaction between a basic cluster and acidic phospholipids (reviewed in Resh, 1999). This "myristate plus basic" motif is found in a number of membrane localized proteins, including src, MARCKS, and HIV-1 Gag (Taniguchi and Manenti, 1993; Sigal *et al.*, 1994; Zhou *et al.*, 1994) and also appears to govern the localization of Nullo to the membrane of the cellularization front.

Nullo is one of ~35 sequences in the *D. melanogaster* genome that contains the MGXXXS/T consensus site for N-terminal myristoylation. Although the majority of these genes have been characterized genetically and developmentally, only a handful have actually been shown to be myristoylated (Neel and Young, 1994; Teng *et al.*, 1994; Rossi *et al.*, 1999), and the in vivo role of the myristoylation site has been studied only in the neural protein Numb (Knoblich *et al.*, 1997). Using a yeast NMT system (Duronio *et al.*, 1990), we demonstrated that Nullo is modified by the addition of a myristoyl group, and this is prevented by the substitution of alanine for glycine in position 2. We believe that this in vitro demonstration of myristoylation is relevant to the in vivo modification of Nullo for two reasons. First, the consensus target for NMT activity appears to be well conserved between *Drosophila* and other organisms. Both *Drosophila* embryonic extracts and Schnieder cells have been shown to have an NMT activity that modifies myristoyl proteins from other organisms in a specific manner, and the corresponding NMT gene has been shown to be expressed before cellularization (Deichaite *et al.*, 1988; Ntwasa *et al.*, 1997; Benting *et al.*, 2000). Second, the glycine to alanine mutation which blocks myristoylation in vitro has a striking effect on the subcellular localization of Nullo in vivo, suggesting that the N-myristoylation site is important for the targeting of Nullo to the plasma membrane.

During metaphase of nuclear cycle 13, Nullo lacking a myristoylation site (ΔM) still shows normal localization to the plasma membrane of the metaphase furrows. However, at the onset of cycle 14, ΔM is lost from the cellularization front and begins to accumulate in the nucleus where it remains until midcellularization. UAS-ΔM expressed during late cellularization showed no membrane association, but was observed in the nucleus. The difference in ΔM localization before and after the onset of cellularization suggests that some aspect of Nullo targeting or membrane association changes at this point. The fact that ΔMP, which lacks both N-terminal domains, shows no early membrane association suggests that the initial association of Nullo with the plasma membrane requires the presence of a positive charge and that the positive charge is no longer sufficient for membrane localization after the onset of cellularization. The electrostatic interaction may be disrupted by changes in the phospholipid content of the apical membrane as new membrane is inserted during cellularization. In contrast, endogenous and ectopic expression of ΔP demonstrates that the myristoylation site alone is sufficient for partial membrane localization throughout cellularization.

After cycle 13, ΔM accumulates in the nucleus, which is surprising, because Nullo does not contain a predicted nuclear localization signal, and we have never observed wild-type Nullo protein in the nucleus. It is possible, however that the basic cluster confers a nuclear localization that is normally overridden by the membrane targeting of the N-terminal myristoyl group. In the absence of the myristoyl group, Nullo would associate with the nucleus via the polybasic region, and as we observed, the association with the nucleus would be diminished by the removal of the positively charged cluster (ΔMP). A similar observation has been made with the Moloney MuLV Gag protein, where mutation of the N-terminal myristoylation site roughly doubles the amount of protein found in the nucleus (Nash *et al.*, 1993).

Although the ΔM, ΔP, and ΔMP proteins all retained some ability to rescue the *nullo* mutant phenotype, only ΔP was able to partially block apical junction formation when expressed ectopically during late cellularization. One explanation for the difference in activity may lie in the levels of functional protein required in each of these assays. As seen with the LII27.32R5 allele, the rescue of the *nullo* phenotype requires levels of Nullo protein much lower than those normally seen at the start of cycle 14. In contrast, the ectopic effects on apical junction formation require at least half the protein level seen at the onset of cellularization. In the case of ΔM the difference in activity may also result from the fact that this protein retains membrane localization during the cycle 13 to cycle 14 transition when basal junctions are formed but lacks any detectable membrane localization when ectopically expressed during late cellularization. The fact that both assays showed a decline in Nullo activity that correlated with the loss of Nullo from the plasma membrane suggests that the "myristate plus basic" motif controls membrane localization and that the membrane association of Nullo is essential for its role in junction formation.

The fact that both ΔM and ΔP retained some ability to associate with the plasma membrane is unusual, as removal of either the myristoyl group or the basic region is usually enough to prevent plasma membrane association in "myristate plus basic" proteins (reviewed in Resh, 1999). Interestingly, mutation of the myristoylation site of Numb also failed to affect membrane localization, although truncation of the first 40 amino acids of the N-terminus caused the protein to accumulate in the cytoplasm (Knoblich *et al.*, 1997). It remains to be seen if Numb contains a bipartite localization motif similar to that seen in Nullo and whether the independent membrane targeting activity of myristoyl groups and basic clusters is a common occurrence in *Drosophila*.

Loss of Nullo has its primary effect on the formation of basal junctions from the small points of membrane contact that arise at the start of cellularization. The results presented in this article argue that Nullo is not an essential component

of the junction itself but is required to stabilize nascent junctions, especially at higher temperatures where the membrane contacts may be more fluid. Ectopic expression of Nullo during late cellularization may also stabilize junctional components as they reach the plasma membrane, but in this case it would prevent the clustering required to form the apical adherens junctions (Hunter and Wieschaus, 2000). The effect of Nullo on adherens junctions is compatible with numerous direct and indirect modes of action. However, the structural studies presented here suggest that Nullo's activity requires its cell-surface localization and therefore may involve a physical interaction between Nullo and some component of the basal junction, or the accompanying cytoskeleton.

ACKNOWLEDGMENTS

The authors thank members of the Wieschaus and Schupbach laboratories for helpful discussions; Jen Zallen, Jorg Grosshans, Jeff Thomas, and two anonymous reviewers for comments on the manuscript; Reba Samanta and Joe Goodhouse for assistance with histology and confocal microscopy; Romy Knittel for technical assistance in cloning and characterizing the *D. virilis* homologue; and Cynthia Hsuan-Hung for maintaining stocks. The authors are grateful to J. Gordon, V. Malhotra, P. Schedl, and D. St. Johnston for providing stocks and reagents. This work was supported by the Howard Hughes Medical Institute grant 5R37HD15587 from the National Institute of Child Health and Human Development and grant 95-00258/3 from the U.S.-Israel Binational Science Foundation.

REFERENCES

Benting, J., Lecat, S., Zacchetti, D., and Simons, K. (2000). Protein expression in Drosophila Schneider cells. Anal. Biochem. *278*, 59–68.

Brand, A.H., and Perrimon, N. (1993). Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. Development *118*, 401–415.

Caccone, A., Moriyama, E.N., Gleason, J.M., Nigro, L., and Powell, J.R. (1996). A molecular phylogeny for the *Drosophila melanogaster* subgroup and the problem of polymorphism data. Mol. Biol. Evol. *13*, 1224–1232.

Deichaite, I., Casson, L.P., Ling, H.P., and Resh, M.D. (1988). In vitro synthesis of pp60v-src: myristylation in a cell-free system. Mol. Cell. Biol. *8*, 4295–4301.

Duronio, R.J., Rudnick, D.A., Johnson, R.L., Linder, M.E., and Gordon, J.I. (1990). Reconstitution of protein N-Myristoylation in *Escherichia coli*. Methods *1*, 253–263.

Eberl, D.F., and Hilliker, A.J. (1988). Characterization of X-linked recessive lethal mutations affecting embryonic morphogenesis in *Drosophila melanogaster*. Genetics *118*, 109–120.

Fares, H., Peifer, M., and Pringle, J.R. (1995). Localization and possible functions of Drosophila septins. Mol. Biol. Cell *6*, 1843–1859.

Field, C.M., and Alberts, B.M. (1995). Anillin, a contractile ring protein that cycles from the nucleus to the cell cortex. J. Cell Biol. *131*, 165–178.

Foe, V.E., and Alberts, B.M. (1983). Studies of nuclear and cytoplasmic behavior during the five mitotic cycles that precede gastrulation in Drosophila embryogenesis. J. Cell Sci. *61*, 31–70.

Hunter, C., and Wieschaus, E. (2000). Regulated expression of nullo is required for the formation of distinct apical and basal adherens junctions in the Drosophila blastoderm. J. Cell Biol. *150*, 391–401.

Ibnsouda, S., Schweisguth, F., de Billy, G., and Vincent, A. (1993). Relationship between expression of serendipity alpha and cellularization of the Drosophila embryo as revealed by interspecific transformation. Development *119*, 471–483.

Ibnsouda, S., Schweisguth, F., Jullien, D., Kucherer, C., Lepesant, J.A., and Vincent, A. (1995). Evolutionarily conserved positive and negative cis-acting elements control the blastoderm-specific expression of the Drosophila serendipity alpha cellularisation gene. Mech. Dev. *49*, 71–82.

Knoblich, J.A. (2000). Epithelial polarity: the ins and outs of the fly epidermis. Curr. Biol. *10*, R791–R794.

Knoblich, J.A., Jan, L.Y., and Jan, Y.N. (1997). The N terminus of the Drosophila Numb protein directs membrane association and actin-dependent asymmetric localization. Proc. Natl. Acad. Sci. USA *94*, 13005–13010.

Lecuit, T., and Wieschaus, E. (2000). Polarized insertion of new membrane from a cytoplasmic reservoir during cleavage of the Drosophila embryo. J. Cell Biol. *150*, 849–860.

Merrill, P.T., Sweeton, D., and Wieschaus, E. (1988). Requirements for autosomal gene activity during precellular stages of *Drosophila melanogaster*. Development *104*, 495–509.

Müller, H.-A.J., and Wieschaus, E. (1996). Armadillo, bazooka, and stardust are critical for early stages in formation of the zonula adherens and maintenance of the polarized epithelium in Drosophila. J. Cell Biol. *134*, 149–163.

Nash, M.A., Meyer, M.K., Decker, G.L., and Arlinghaus, R.B. (1993). A subset of Pr65gag is nucleus associated in murine leukemia virus-infected cells. J. Virol. *67*, 1350–1356.

Neel, V.A., and Young, M.W. (1994). Igloo, a GAP-43-related gene expressed in the developing nervous system of Drosophila. Development *120*, 2235–2243.

Nicklas, J.A., and Cline, T.W. (1983). Vital genes that flank Sex-lethal, and X-linked sex-determining gene of *Drosophila melanogaster*. Genetics *103*, 617–631.

Ntwasa, M., Egerton, M., and Gay, N.J. (1997). Sequence and expression of Drosophila myristoyl-CoA: protein N-myristoyl transferase: evidence for proteolytic processing and membrane localization. J. Cell Sci. *110*, 149–156.

O'Neil, M.T., and Belote, J.M. (1992). Interspecific comparison of the transformer gene of Drosophila reveals an unusually high degree of evolutionary divergence. Genetics *131*, 113–128.

Oda, H., Tsukita, S., and Takeichi, M. (1998). Dynamic behavior of the cadherin-based cell-cell adhesion system during Drosophila gastrulation. Dev. Biol. *203*, 435–450.

Oda, H., Uemura, T., Shiomi, K., Nagafuchi, A., Tsukita, S., and Takeichi, M. (1993). Identification of a Drosophila homologue of alpha-catenin and its association with the armadillo protein. J. Cell Biol. *121*, 1133–1140.

Postner, M.A., and Wieschaus, E.F. (1994). The nullo protein is a component of the actin-myosin network that mediates cellularization in *Drosophila melanogaster* embryos. J. Cell Sci. *107*, 1863–1873.

Resh, M.D. (1999). Fatty acylation of proteins: new insights into membrane targeting of myristoylated and palmitoylated proteins. Biochim. Biophys. Acta *1451*, 1–16.

Riggleman, B., Schedl, P., and Wieschaus, E. (1990). Spatial expression of the Drosophila segment polarity gene armadillo is posttranscriptionally regulated by wingless. Cell *63*, 549–560.

Ripoche, J., Link, B., Yucel, J.K., Tokuyasu, K., and Malhotra, V. (1994). Location of Golgi membranes with reference to dividing nuclei in syncytial Drosophila embryos. Proc. Natl. Acad. Sci. USA *91*, 1878–1882.

Rose, L.S., and Wieschaus, E. (1992). The Drosophila cellularization gene nullo produces a blastoderm-specific transcript whose levels respond to the nucleocytoplasmic ratio. Genes Dev. *6*, 1255–1268.

Rossi, E.A., Li, Z., Feng, H., and Rubin, C.S. (1999). Characterization of the targeting, binding, and phosphorylation site domains of an A kinase anchor protein and a myristoylated alanine-rich C kinase substrate-like analog that are encoded by a single gene. J. Biol. Chem. *274*, 27201–27210.

Schejter, E.D., and Wieschaus, E. (1993). bottleneck acts as a regulator of the microfilament network governing cellularization of the Drosophila embryo. Cell *75*, 373–385.

Sigal, C.T., Zhou, W., Buser, C.A., McLaughlin, S., and Resh, M.D. (1994). Amino-terminal basic residues of Src mediate membrane binding through electrostatic interaction with acidic phospholipids. Proc. Natl. Acad. Sci. USA *91*, 12253–12257.

Simpson, L., and Wieschaus, E. (1990). Zygotic activity of the nullo locus is required to stabilize the actin-myosin network during cellularization in Drosophila embryos. Development *110*, 851–863.

Spradling, A.C. (1986). P-element mediated transformation. In: Drosophila: A Practical Approach, ed. D. B. Roberts, Oxford: IRL Press, 175–197.

Taniguchi, H., and Manenti, S. (1993). Interaction of myristoylated alanine-rich protein kinase C substrate (MARCKS) with membrane phospholipids. J. Biol. Chem. *268*, 9960–9963.

Tautz, D., and Pfeifle, C. (1989). A non-radioactive in situ hybridization method for the localization of specific RNAs in Drosophila embryos reveals translational control of the segmentation gene hunchback. Chromosoma *98*, 81–85.

Teng, D.H., Chen, C.K., and Hurley, J.B. (1994). A highly conserved homologue of bovine neurocalcin in *Drosophila melanogaster* is a Ca(2+)-binding protein expressed in neuronal tissues. J. Biol. Chem. *269*, 31900–31907.

Thummel, C.S., and Pirotta, V. (1991). Technical notes: new CaSpeR P-element vectors. *Drosophila* Information Newsletter 1991 (2).

Vallejo, A.N., Pogulis, R.J., and Pease, L.R. (1995). Mutagenesis and synthesis of novel recombinant genes using PCR. In: PCR Primer: A Laboratory Manual, ed. C.W. Dieffenbach, G.A.Dveksler, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 603–612.

Vincent, A., Colot, H.V., and Rosbash, M. (1985). Sequence and structure of the serendipity locus of *Drosophila melanogaster*. A densely transcribed region including a blastoderm-specific gene. J. Mol. Biol. *186*, 149–166.

Warn, R.M., and Robert-Nicoud, M. (1990). F-actin organization during the cellularization of the Drosophila embryo as revealed with a confocal laser scanning microscope. J. Cell Sci. *96*, 35–42.

Wieschaus, E., Nusslein-Volhard, C., and Jurgens, G. (1984). Mutations affecting the pattern of the larval cuticle in *Drosophila melanogaster*. III. Zygotic loci on the X chromosome and fourth chromosome. Wilhem Roux's Arch. Dev. Biol. *193,* 296–307.

Wieschaus, E., and Sweeton, D. (1988). Requirements for X-linked zygotic activity during cellularization of early Drosophila embryos. Development *104*, 483–493.

Young, P.E., Pesacreta, T.C., and Kiehart, D.P. (1991). Dynamic changes in the distribution of cytoplasmic myosin during Drosophila embryogenesis. Development *111*, 1–14.

Zhou, W., Parent, L.J., Wills, J.W., and Resh, M.D. (1994). Identification of a membrane-binding domain within the amino-terminal region of human immunodeficiency virus type 1 Gag protein which interacts with acidic phospholipids. J. Virol. *68*, 2556–2569.