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The *Drosophila* centrosomal protein Nuf is required for recruiting Dah, a membrane associated protein, to furrows in the early embryo

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SUMMARY

During mitosis of the *Drosophila* cortical syncytial divisions, actin-based membrane furrows separate adjacent spindles. Our genetic analysis indicates that the centrosomal protein Nuf is specifically required for recruitment of components to the furrows and the membrane-associated protein Dah is primarily required for the inward invagination of the furrow membrane. Recruitment of actin, anillin and peanut to the furrows occurs normally in *dah*-derived embryos. However, subsequent invagination of the furrows fails in *dah*-derived embryos and the septins become dispersed throughout the cytoplasm. This indicates that stable septin localization requires Dah-mediated furrow invagination. Close examination of actin and Dah localization in wild-type embryos reveals that they associate in adjacent particles during interphase and co-localize in the invaginating furrows during prophase and metaphase. We show that the Nuf centrosomal protein is required for recruiting the membrane-associated protein Dah to the furrows. In *nuf*-mutant embryos, much of the Dah does not reach the furrows and remains in a punctate distribution. This suggests that Dah is recruited to the furrows in vesicles and that the recruiting step is disrupted in *nuf* mutants. These studies lead to a model in which the centrosomes play an important role in the transport of membrane-associated proteins and other components to the developing furrows.

Key words: *Drosophila*, Centrosome, Septin, Membrane, Actin, Embryo

INTRODUCTION

Invagination of the cleavage furrow is thought to involve an actomyosin-based contractile ring that pinches off the daughter cells (for reviews see Fishkind and Wang, 1995; Glotzer, 1997). Membrane addition during cleavage furrow invagination is also necessary to accommodate the increased surface area required of the daughter cells. In early cleavage stage *Xenopus* embryos, new membrane is generated through vesicle fusion (Bluemink and de Laat, 1973; Byers and Armstrong, 1986). In cellularizing *Drosophila* embryos, considerable new membrane is necessary for furrowing (Fullilove and Jacobson, 1971). Ultrastructural analysis of cellularizing *Drosophila* embryos identifies coated pits and multilamellar bodies as likely sources of the new membrane (Fullilove and Jacobson, 1971; Sanders, 1975; Bluemink et al., 1985).

Analysis of the four nuclear divisions preceding cellularization of the early *Drosophila* embryo provides an excellent system to study furrow formation (Sullivan and Theurkauf, 1995). The early *Drosophila* embryo undergoes thirteen rapid synchronous nuclear divisions that occur in the absence of cytokinesis (Zalokar and Erk, 1976; Foe and Alberts, 1983). The first nine divisions occur in the interior of the embryo whereas division cycles 10-13 take place at the embryonic periphery. Cytoskeletal organization during the cortical nuclear divisions has been well characterized (Warn et al., 1984; Karr and Alberts, 1986; Foe et al., 1993). At interphase, actin caps form above each cortical nucleus and its apically positioned centrosome pair. As the nuclei progress through prophase the centrosomes migrate toward opposite poles of the nucleus and the actin caps undergo a dramatic redistribution to form plasma membrane-based furrows that separate neighboring spindles. These furrows are maintained through metaphase and regress as the nuclei progress into telophase. Actin caps once again form in the following interphase.

Centrosomes play an important role in directing these cortical cytoskeletal rearrangements. For example, free centrosomes, unassociated with a nucleus, induce actin-dependent cell-cycle rearrangements (Raff and Glover, 1989; Yasuda et al., 1991). Further evidence comes from observations of the maternal-effect mutation *daughterless-abo-like (dal)*, which disrupts centrosome separation and consequently centrosome spacing during metaphase (Sullivan et al., 1990, 1993). In these embryos, actin fails to redistribute into furrows in regions in which there is an abnormally large distance between neighboring centrosomes.

Insight into how centrosomes direct furrow formation has come from studies of the maternal-effect *Drosophila* mutation,

nuclear-fallout (nuf) (Sullivan et al., 1993; Rothwell et al., 1998). *nuf* encodes a protein that concentrates at the centrosomes during prophase and is cytoplasmic throughout the rest of the nuclear cycle (Rothwell et al., 1998). The transient nature of Nuf centrosomal localization suggests that it is not a core centrosomal component. The absence of Nuf does not affect microtubule dynamics (Sullivan et al., 1993). However, actin is not properly recruited to the furrows during prophase and remains abnormally concentrated at the centrosomes (Rothwell et al., 1998). This results in the formation of partial furrows with breaks in regions farthest from the centrosomes. Nuf may therefore function to direct the recruitment of actin from the centrosomes to the metaphase furrows. Alternatively, the partial furrow phenotype in *nuf* mutants may reflect limited activity of a Nuf-containing complex. Interestingly, Nuf is not responsible for the recruitment of the conserved furrow components, anillin and peanut (Rothwell et al., 1998).

discontinuous actin hexagon (dah), is a maternal-effect *Drosophila* mutation that produces a mutant phenotype similar to *nuf*; the interphase actin caps form normally but the metaphase furrows are disrupted (Zhang et al., 1996). *dah* encodes a protein that has some sequence homology to dystrophin, the mammalian gene required for maintaining muscle integrity (for review see Ahn and Kunkel, 1993; Zhang et al., 1996). Our analysis of the *dah* mutant maternal-effect phenotype demonstrates that failure of the metaphase furrows to invaginate during the prophase to metaphase transition is the primary defect in these embryos. Recently, biochemical analysis has shown that Dah is a membrane associated protein (C. Zhang, submitted). Here we show that Dah exhibits a punctate staining pattern during interphase and early in prophase these particles localize to the future furrow regions. Our co-localization studies demonstrate that these punctate particles contain membrane. As prophase proceeds Dah localizes to the invaginating furrows. At metaphase the majority of Dah is co-localized with actin along the furrow. During prophase in *nuf*-derived embryos, much but not all of the Dah fails to localize to the newly forming furrows. The mislocalized Dah in *nuf* maintains its association with the membrane-containing particles. This suggests that Nuf is required for the recruitment of Dah-containing membrane to furrows.

Although its localization is disrupted in *nuf*-mutants, Dah protein is still present and a portion of it distributes into the furrow. If this furrow also contains actin, furrow invagination proceeds. This often results in the formation of partial, broken furrows adjacent to the centrosomes. Here we show that the free ends of disrupted furrows in *nuf*-derived embryos extend to an equal depth as intact furrows indicating that this process is not solely contractile and includes an alternative mechanism such as vesicle fusion. These studies lead to a model in which the centrosome plays a key role in recruiting new membrane to the developing furrows.

MATERIALS AND METHODS

Drosophila stocks

The isolation and initial characterization of the *nuf* and *dah* mutations has been previously described (Sullivan et al., 1993; Zhang et al., 1996; Rothwell et al., 1998). Oregon-R served as the wild-type control stock (Lindsley and Zimm, 1992). All of the experiments described in this manuscript used null alleles of *nuf (nuf^{1})* and *dah* (Sullivan et al., 1993; Zhang et al., 1996; Rothwell et al., 1998). That these are null alleles is supported by immunological (*nuf* and *dah*) as well as genetic and molecular biological *(nuf)* analyses. The stocks were maintained on standard corn meal/molasses medium.

Fixation and immunofluorescence

Immunofluorescence analysis was performed as described by Karr and Alberts (1986). Actin was stained with fluorescently labeled phalloidin on formaldehyde fixed hand devitellinized embryos. Embryos were dechorionated in 50% Clorox bleach solution and fixed in a heptane solution saturated with 37% formaldehyde for 40 minutes at room temperature. The fixed embryos were then transferred to a small piece of Whatmann paper and the heptane allowed to evaporate. These embryos were transferred to double stick tape in the lid of a small Petri dish (35 × 10 mm) and covered with PBTA buffer (1× PBS, 1% BSA, 0.05% Triton X-100, 0.02% NaAzide). The vitelline membranes were removed by hand under a dissecting microscope using a 23 gauge needle on a 3 ml syringe.

To view the DNA, embryos were incubated in 10 mg/ml RNase for 2 hours at 37°C, followed by extensive rinsing in PBS and mounted in a 90% glycerol, PBS solution containing 1 mg/ml *N-N*-1-4-phenylenediamine and 1 μg/ml propidium iodide. Immunofluorescence analyses using the rabbit anti-anillin (Field and Alberts, 1995), anti-peanut (Field et al., 1996) and anti-Dah (Zhang et al., 1996) antibodies and the mouse anti-phosphotyrosine antibody (Santa Cruz Biotechnology, Santa Cruz, CA) were performed on formaldehyde fixed hand devitellinized embryos as described above. Secondary anti-mouse and anti-rabbit antibodies tagged with rhodamine, fluorescein, or Cy5 were applied to the embryos as described by Karr and Alberts (1986). Nuclear cycle stages were determined by staining embryos with DAPI (4,6-diaminido-2-phenylindole).

Microscopy was performed using an Olympus IMT2 inverted photoscope equipped with a Bio-Rad MRC600 laser confocal imaging system and a Leitz DMIRB inverted photoscope equipped with a Leica TCS NT laser confocal imaging system. The cortical nuclear cycles (10-14) were determined by using the Bio-Rad and Leica imaging software to estimate nuclear densities.

RESULTS

Actin and Dah exhibit a punctate staining pattern during interphase and a furrow localization during metaphase

Previous studies demonstrate that Dah co-localizes with actin and concentrates in the metaphase furrows (Zhang et al., 1996). Here we extend these studies by closely examining the relationship between Dah and actin localization throughout the process of metaphase furrow formation**.** At interphase, actin is present throughout the embryo but is concentrated in caps above each nucleus. Both Dah and actin exhibit a punctate staining pattern at this time (Fig. 1A and E). Dah staining particles (red) appear to lie side by side with actin-containing particles (green) with the yellow coloring depicting regions of overlap. This pattern is more pronounced in regions between the nuclei and below the caps (Fig. 1A and E, see insets).

During the prophase to metaphase transition, the actin undergoes a dramatic rearrangement and concentrates at the newly formed furrows between each nucleus. Initially, the side by side punctate staining of Dah and actin is maintained (Fig.

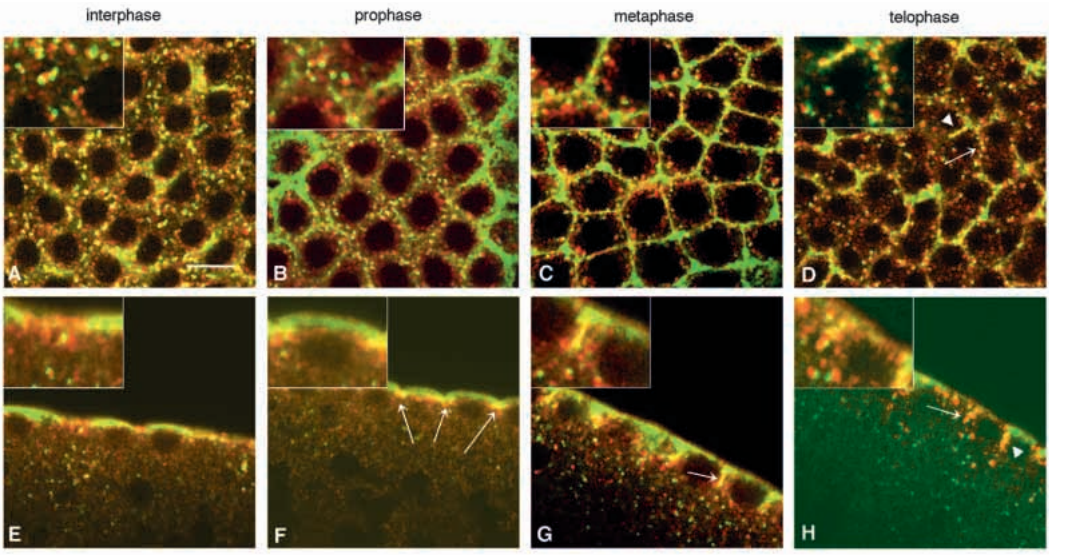

Fig. 1. Localization of Dah. Images of wild-type embryos double stained for F-actin (phalloidin, green, fluorescein) and Dah (red, Cy5). Surface (A-D) and sagital (E-H) views are shown for nuclear cycle 12 embryos in interphase (A,E), prophase (B,F), metaphase (C,G) and telophase (D,H). Embryos were prepared by hand devitellinization and stained as described in Materials and Methods. See text for details. Inset, ×2 magnification. Bar, 10 μm.

1B, inset) and Dah is concentrated at the leading edge of the developing furrows (Fig. 1F, see arrows). At metaphase, Dah becomes concentrated in the metaphase furrows along with the actin (Fig. 1C and G). At this time, Dah and actin exhibit either a side-by-side punctate pattern in the cytoplasm or co-localize along the length of the invaginating furrow (Fig. 1G, see arrow and inset).

At telophase, Dah and actin remain attached in some regions of the furrows (Fig. 1D and H, see arrowheads and insets) while other regions contain free actin/Dah particles (Fig. 1D and H, see arrows and insets). This suggests that actin/Dah containing particles are released as the furrows break down and caps begin to reform.

During the entire division cycle, a subset of Dah protein remains in a perinuclear distribution and does not appear to associate with actin particles (red solo dots surrounding the nuclei in upper panels). The punctate actin particles, however, appear to consistently associate with the Dah particles. The significance of the actin-Dah association is unclear but may represent a step in which actin first becomes associated with membrane.

Dah is required for furrow extension

The absence of Dah results in disruption of the metaphase furrows indicating that Dah plays a critical role in furrow organization (Zhang et al., 1996). Here, we closely examine the prophase to metaphase transition in *dah*-derived embryos to define the specific stage of furrow formation affected (Fig. 2). We find that the initiation of furrow formation at prophase is unaffected in *dah*-derived embryos. Anillin and peanut, a *Drosophila* septin, are conserved components of metaphase and cytokinesis furrows that serve as early markers of furrow formation (Miller et al., 1989; Neufeld and Rubin, 1994; Fares et al., 1995; Field and Alberts, 1995; Rothwell et al., 1998). In wild-type embryos, furrow formation initiates at prophase with the recruitment of anillin, peanut and actin to the metaphase furrows. During prophase in wild-type embryos, all of these components are co-localized in the newly developing metaphase furrows (Fig. 2, top row). An equivalent series of images from *dah*-derived embryos indicate that, with respect to actin, anillin and peanut localization, prophase occurs normally in *dah*-derived embryos (Fig. 2, second row).

Although furrow initiation is normal, invagination of the furrows is disrupted in *dah*-mutant embryos. In normal metaphase embryos, actin, anillin and peanut exhibit extensive co-localization (Fig. 2, middle row). The side views of these same embryos indicate that the furrows have invaginated and that peanut and anillin are concentrated at the leading edge of these furrows. The bottom set of panels shows a series of equivalent metaphase images for *dah*-derived embryos. As previously reported, surface views demonstrate extensive disruptions in the network of actin staining (Zhang et al., 1996). Images taken just below the embryo surface reveal an uneven actin distribution (Fig. 2, bottom row). However, actin that has been recruited to the furrows appears stable. Although there is some variation in the phenotype, observation of sagital views of many embryos reveals that the major defect is a failure of furrow extension. Even in the regions in which the actin network is intact, furrow extension does not occur. In contrast, *nuf*-derived embryos exhibit extensive disruptions in their actin network but furrow extension occurs (Rothwell et al., 1998; Fig. 3).

These images also demonstrate that the *dah*-mutant differentially affects anillin and peanut localization. Anillin and actin co-localize in the *dah* mutation. Anillin localization fails only in those regions in which actin localization fails. In contrast, even in those regions in which actin is properly localized, peanut localization fails.

Recruitment of Dah to the metaphase furrows requires the Nuf centrosomal protein

Nuf is a protein that localizes to the centrosomes during prophase, when actin and other components are being localized to the furrows (Rothwell et al., 1998). In the absence of Nuf, actin is not properly recruited to the furrows. Interestingly, Nuf is not required for the recruitment of two other conserved furrow components peanut and anillin (Rothwell et al., 1998). To determine whether Nuf is required for the recruitment of Dah to the metaphase furrows, we examined Dah localization in *nuf*-derived embryos. Fig. 3 depicts wild-type and *nuf*-

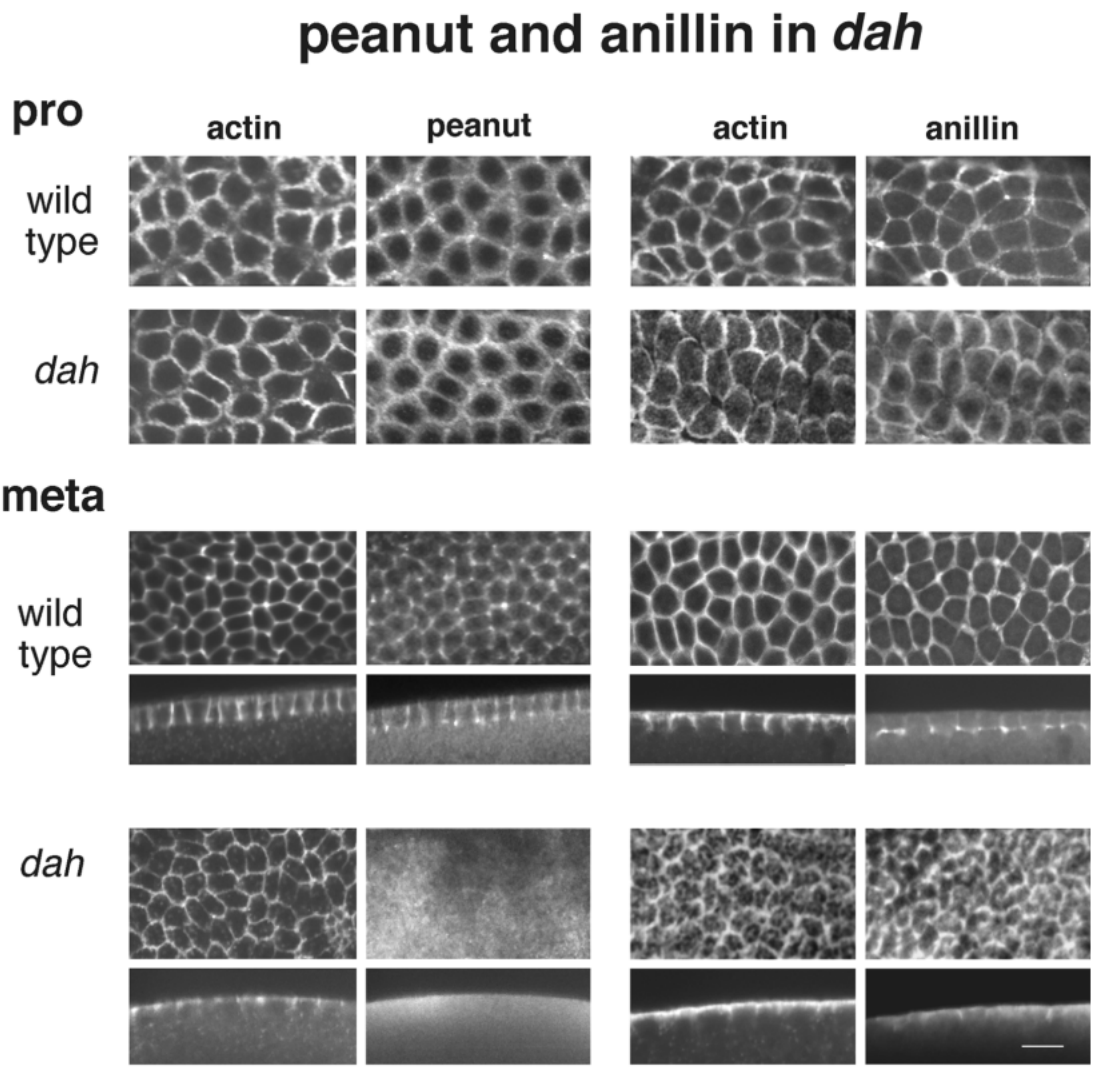

Fig. 2. Dah is required for furrow extension. Localization of furrow components in wild-type and *dah*-derived embryos at prophase (top panels-surface views) and metaphase (bottom panels-surface and sagital views) of nuclear cycles 12 and 13. Left hand panels depict embryos double stained for actin (phalloidin) and peanut (a *Drosophila* septin). Right hand panels depict embryos double stained for actin (phalloidin) and anillin. *dah*-mutant embryos are specifically disrupted in furrow elongation. Bar, 10 μm.

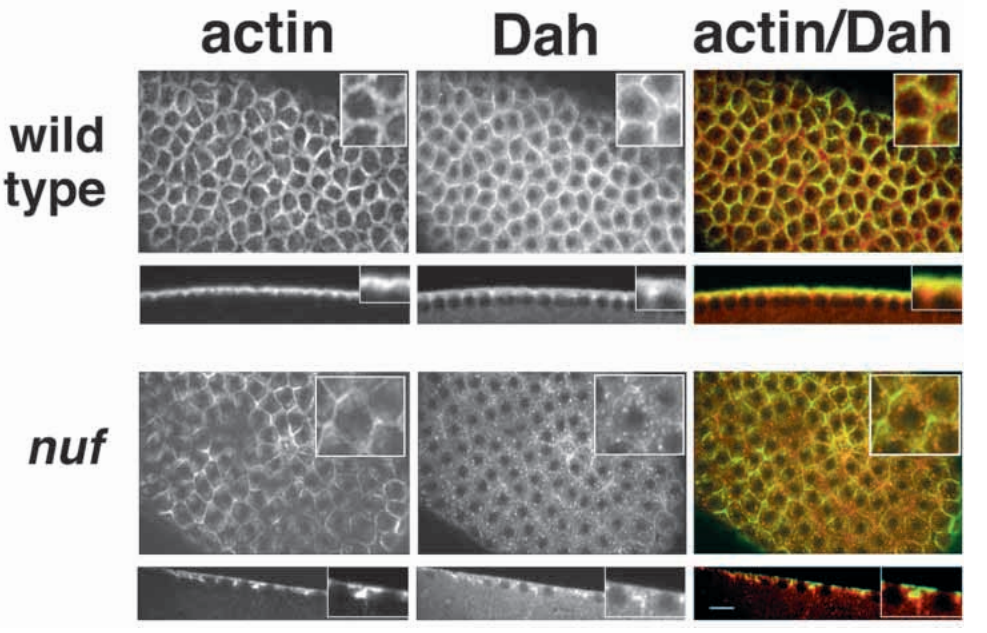

Fig. 3. Dah is mislocalized in *nuf*-derived embryos. Wild-type and *nuf*-mutant embryos double stained for actin (phalloidin) and Dah. Embryos are in prophase of nuclear cycle 12. Merged images show relative localizations for actin (green) and Dah (red). Dah is mislocalized in *nuf*-mutants and gives a punctate staining pattern. Inset, ×2 magnification. Bar, 10 μm.

derived embryos double-stained for actin (green) and Dah (red). During prophase in the normal embryo, actin and Dah localize to the newly forming metaphase furrows with Dah concentrating at the furrow tips (Fig. 3, inset). During prophase in *nuf*-derived embryos, there are large regions in which actin localization has failed (Rothwell et al., 1998; Fig. 3). This image also reveals that Dah localization to the furrows is severely disrupted and remains in a punctate pattern. Although Dah localization is severely disrupted, there are always small regions in which Dah localizes to the furrows. Significantly, in these regions furrows appear to have undergone extension (Fig. 3, inset).

Dah is associated with membrane-containing particles during prophase

The images described in the previous sections suggest that Dah may localize with membrane. To examine a possible association between Dah and membrane, we performed co-localization studies with anti-phosphotryrosine and anti-Dah antibodies. Tyrosine-phosphorylated proteins are enriched in membrane; therefore, antibodies directed against phosphotyrosines serve as excellent membrane markers (Eiseman and Kinsey, 1982; Dasgupta and Garbers, 1983; Heldin and Westermark, 1984; Sullivan, 1987; Piedimonte et al., 1988; Allen and Aderem, 1995). As described in the previous section, Dah exhibits a punctate pattern in regions in which the furrows are developing (Figs 1 and 4). The anti-phosphotyrosine clearly stains the plasma membrane in the newly formed furrows and also produces a punctate staining pattern (Fig. 4). Dah and phosphotyrosine exhibit extensive co-localization with respect to this punctate staining distribution (Fig. 4, surface view). The majority of these doubly stained punctate dots occur in regions just below the invaginating furrows. Dah and phosphotyrosinated proteins also show extensive co-localization at the tips of the furrows, regions where the Dah concentration is highest. Unlike actin, Dah is not concentrated in the apical plasma membrane.

To determine whether the mislocalized Dah in *nuf*-derived embryos is maintained in membrane containing particles, we double stained *nuf*-derived embryos with antibodies directed against Dah and phosphotyrosine. This experiment revealed that Dah and phosphotyrosinated proteins co-localize in a punctate pattern in *nuf*-derived embryos (Fig. 5).

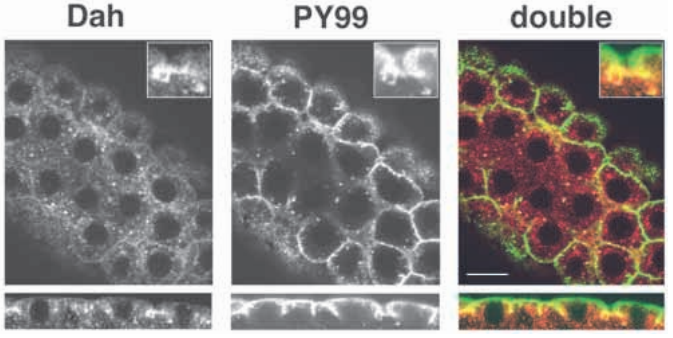

Fig. 4. Co-localization of Dah with plasma membrane. Surface and sagital views of a wild-type embryo in prophase of nuclear cycle 11 double stained for Dah (red) and phosphotyrosine (py99, green). Dah and py99 co-localize in a punctate pattern in a region ahead of the invaginating furrows and appear to concentrate at the furrow tips. Unlike actin, Dah is not concentrated in the apical plasma membrane. Co-localization of Dah and phosphotyrosine results in a yellow staining. Inset, ×2 magnification. Bar, 10 μm.

In *nuf*-derived embryos, the free ends of incomplete metaphase furrows extend normally

The above studies suggest that incorporation of new membrane may be a means of furrow extension during the prophase to metaphase transition. This implies that the process is not solely contractile. To test this idea, we examined the depth to which extension occurs in disrupted furrows possessing free ends. If extension requires long range contractile interactions, furrows with free ends should not be able to extend as deeply as intact furrows. The presence of both broken and intact furrows in *nuf*-mutant embryos allows one to compare the depth of complete furrows with the depth obtained by free ends of incomplete furrows positioned close by. This is what was done in Fig. 6, a series of images of a *nuf*-derived embryo during prometaphase of the cortical divisions double-stained for actin (green) and nuclei (red). These images are taken at 1.0 μm intervals beginning just beneath the plasma membrane and extending into the interior of the embryo to a final depth of 4.0 μm. The arrowhead and arrow depict an intact closed ring and a furrow ending in a free end, respectively. The free furrow end extends to the same depth as the region of the furrow that is part of a closed ring structure. This adds further support to the idea that this process relies on non contractile mechanisms such as membrane addition.

DISCUSSION

Mutational analysis identifies distinct steps in the process of furrow formation

The two mutations described here, *nuf* and *dah*, identify distinct steps in the process of metaphase furrow formation in syncytial blastoderm embryos. During the initial stage, actin and other components are recruited to the furrow regions. Following this, furrow invagination occurs. We demonstrate that Nuf is specifically involved in recruiting components to the furrow while Dah is required for furrow invagination. In *nuf*-mutants, actin recruitment to regions distant from the centrosomes often fails. This results in the inability of furrows to form in these regions (Rothwell et al., 1998). Alternatively, the partial formation of furrows in the *nuf* null mutation could be due to incomplete activity of a Nuf-containing multi-protein complex. Furrow regions closest to the centrosomes, in which actin and the other furrow components are properly recruited, undergo normal invagination. This indicates that Nuf is specifically required for recruitment of furrow components and is not involved in the subsequent stages of furrow formation. In contrast, recruitment of furrow components occurs normally in *dah*-derived embryos but furrow extension fails. Thus, Dah functions primarily in the invagination process. It is likely that other cortical components will fall into one of these classes or play roles in other distinct steps in furrow formation. For example, the unconventional myosin, 95F-myosin, appears to play a role in the invagination process; injection of antibodies directed against 95F myosin results in metaphase furrows that do not invaginate as deeply as those in normal embryos (Mermall and Miller, 1995).

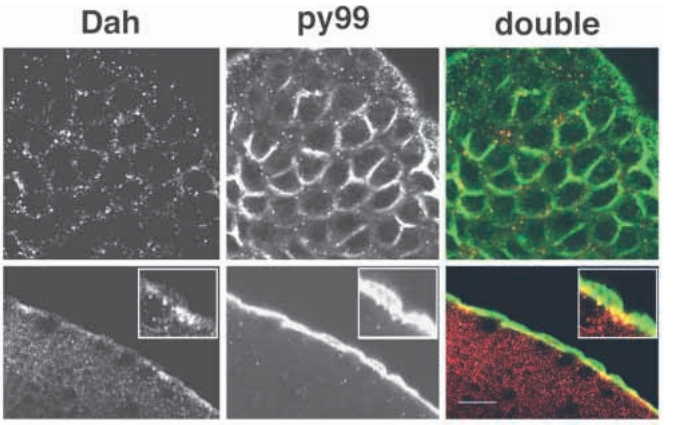

Fig. 5. Mislocalized Dah in *nuf*-mutants is maintained in vesicles. *nuf*-derived embryo in early prophase of nuclear cycle 12 double stained for Dah and phosphotyrosine (py99). Surface and sagital views are shown along with a merged image of phosphotyrosine (green) and Dah (red). Co-localization of Dah and phosphotyrosine results in a yellow staining. Inset, ×2 magnification. Bar, 10 μm.

Mechanisms of furrow formation

During cytokinesis, the cleavage furrow contracts through an actomyosin based mechanism (for reviews see Fishkind and Wang, 1995; Glotzer, 1997). Actomyosin based contraction may also play a role in formation of the metaphase and cellularization furrows of the early *Drosophila* embryo. Myosin II is present at the tips of both the invaginating metaphase and cellularization furrows indicating that invagination may involve contractile processes (Young et al., 1991; Field and Alberts, 1995). Myosin II localizes to metaphase furrows specifically during membrane invagination, leaving the furrow tips once they are fully formed (Field and Alberts, 1995; our unpublished observations). Injection of anti-myosin II antibodies into syncytial embryos disrupts cortical nuclear organization and inhibits subsequent cellularization (Kiehart, 1990).

Several lines of evidence indicate that formation of cellularization furrows also requires membrane addition. The plasma membrane above each nucleus contains microvilli-like projections that increase in number during the initial, slow phase of cellularization and disappear in the later fast phase (Fullilove and Jacobson, 1971). Therefore, the early phase of cellularization may involve membrane recruitment at the cell surface for microvilli formation while the fast phase utilizes the excess membrane for invagination. However, calculations indicate that the membrane supplied by these microvilli-like projections is not sufficient to complete cellularization (Fullilove and Jacobson, 1971). Therefore, other mechanisms of membrane addition may be involved in the cellularization process. In support of this, coated pits and multilamellar bodies decorate the furrows (Fullilove and Jacobson, 1971; Sanders, 1975; Bluemink et al., 1985). Some EM studies describe cellularization as a process involving vesicle alignment at the future furrow site followed by their fusion to form double membranes (Loncar and Singer, 1995). The authors suggest that the slow and fast phases utilize different vesicle populations. Zygotic mutations that specifically effect the slow or fast phases support the idea that they occur through different mechanisms (Merrill et al., 1988; Schweisguth et al., 1991).

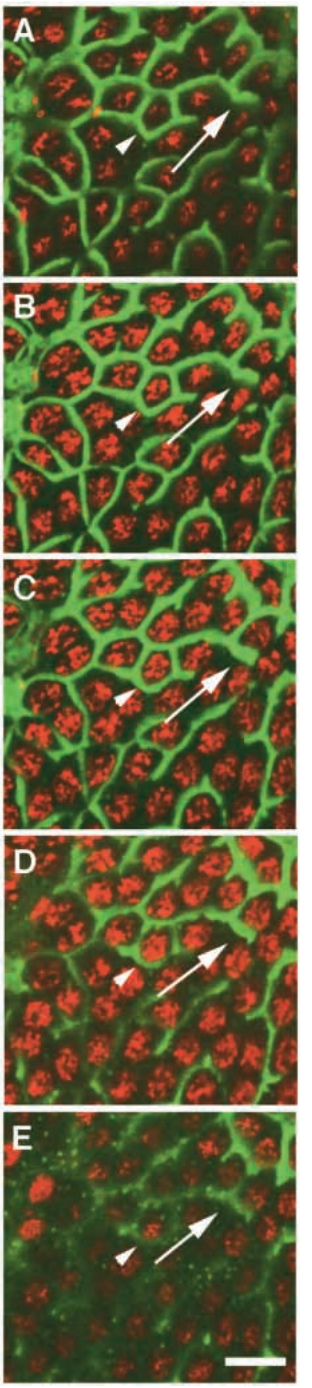

Fig. 6. Incomplete furrows extend in *nuf*-derived embryos. Images of a *nuf*-derived embryo in prometaphase of nuclear cycle 13 double stained for actin (phalloidin-green) and nuclei (red). (A-E) Images taken at 1.0 μm intervals into the embryo starting just below the plasma membrane to a final depth of 4.0 μm. See text for details. Bar, 10 μm.

Genetic studies also support the idea that cellularization requires significant membrane addition. Syntaxins are a family of membrane proteins that are thought to provide specificity for targeting of vesicles to specific membrane compartments (for review see Sollner and Rothman, 1994). Germline clones of the *Drosophila* homologue of syntaxin produce extensive defects in cellularization (Burgess et al., 1997). The *Drosophila* temperature-sensitive mutation *shibire* disrupts the gene encoding dynamin, a protein required for endocytosis (van der Bliek and Meyerowitz, 1991; for review see McNiven, 1998). In addition to neuronal defects, *shibire* also disrupts cellularization (Swanson and Poodry, 1981). At the restrictive

temperature, cellularization furrows do not form and vesicles accumulate in the cytoplasm (Swanson and Poodry, 1981). These data suggest that processes related to endocytosis are required for cellularization.

In these studies, we provide evidence that metaphase furrows, a process very similar to cellularization, may also form through significant addition of membrane. Previous studies demonstrated that Dah is a furrow component required for metaphase furrow formation and cellularization (Zhang et al., 1996). Recently, biochemical analysis demonstrates that Dah is membrane-associated protein (C. X. Zhang et al., unpublished). In the absence of Dah, although recruitment of furrow components occurs normally, furrow invagination fails. In addition, Dah localizes to membrane containing particles that often concentrate at the leading edge of the furrows. These results suggest that invagination of the metaphase furrows occurs through membrane addition and that Dah is a membrane-associated protein required for this process (Fig. 7).

95F-myosin has also been hypothesized to play a role in recruiting particles necessary for furrow formation (Mermall et al., 1994; Mermall and Miller, 1995). The phenotype of embryos injected with antibodies against 95F-myosin is extremely similar to that of *dah*-mutant embryos and the protein products of these genes share similar localization patterns (Mermall and Miller, 1995; Zhang et al., 1996; Fig. 1). Therefore, 95F-myosin and Dah may act together in the process of furrow extension. An attractive hypothesis is that 95F-myosin delivers vesicles containing Dah and other necessary furrow components to the furrow region.

nuf-derived embryos form incomplete furrows (Sullivan et al., 1993; Rothwell et al., 1998). To determine the role of contraction in metaphase furrow formation, we examined invagination of the free ends of these incomplete furrows. Our analysis demonstrates that the free ends of metaphase furrows are stable and invaginate to the same extent as intact normal furrows. This result would not be expected if long range actomyosin-based contraction played a major role in furrow formation. This result is consistent with alternative mechanisms based on membrane addition. One potential mechanism is vesicle fusion.

The role of the centrosome in furrow formation

The centrosome plays a key role in the formation of the actin caps and the metaphase furrows in the early *Drosophila* embryo (Raff and Glover, 1989; Sullivan et al., 1990; Yasuda et al., 1991). The centrosomal protein, Nuf, has been shown to be required for proper actin recruitment to the furrows (Rothwell et al., 1998). Here, we demonstrate that Nuf is also involved in recruiting membrane to the furrows. In wild-type embryos, membrane-bearing particles containing the Dah protein are recruited to the site of furrow formation as the furrows initiate formation during prophase. In similarly staged *nuf*-mutant embryos, many of these membrane containing particles are not properly recruited and remain in a perinuclear punctate distribution. Close examination of Dah and actin localization in wild-type embryos reveals that they localize to adjacent particles that lie between the nuclei and concentrate ahead of the furrow tips (Fig. 1). At metaphase, the majority of Dah and actin co-localize in the furrows. Since Dah is not required for proper actin recruitment to the furrows, it is likely that Dah itself does not play a role in the transport mechanism.

A model consistent with these observations is that Nuf acts at the centrosome to initiate the transport of vesicle-associated components along the microtubules to the furrow regions (Fig. 7). This model accounts for the observation that furrow formation fails primarily in those regions most distant from the centrosomes in which the greatest demand would be placed on the transport process (Rothwell et al., 1998). Similar models have been proposed for membrane transport along the cytoskeleton in other systems. Long range transport of vesicles is thought to utilize kinesins and occur along microtubules; once at the cell periphery, the vesicles are transferred to actin filaments and actin-based motors carry them to their final destinations at the plasma membrane (Langford, 1995; Bi et al., 1997). The recent finding that a microtubule-based motor (conventional kinesin) and an actin motor (myosin V) directly interact is providing insight into the mechanism whereby the

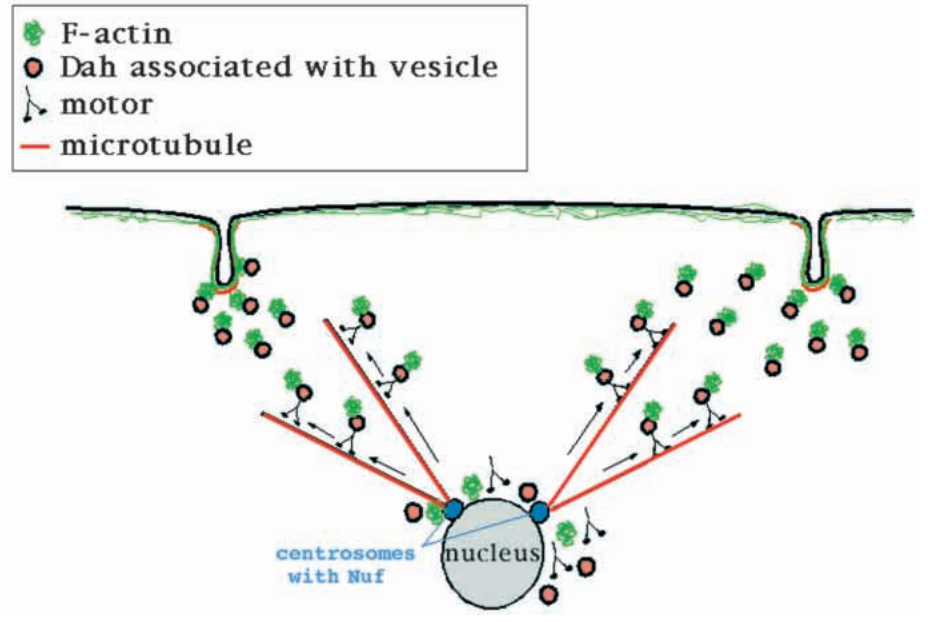

Fig. 7. A model proposing that Nuf acts at the centrosome to load vesicles for transport to the cortex. Side by side particles of actin and Dah are shown being transported together, as a unit. Furrow invagination may occur through vesicle fusion in a process requiring the Dah membrane-associated protein.

same vesicle can move along different cytoskeletal tracks (Huang et al., 1999; Schliwa, 1999). Transport of vesicles necessary for furrow formation in the early *Drosophila* embryo may also occur through coordinated microtubule and actin-based transport systems. In support of this model, drug studies show that disruption of microtubules by injection of colchicine into *Drosophila* embryos halts transport of particles to the cellularization furrows resulting in inhibition of membrane invagination (Foe and Alberts, 1983). In addition, injection of antibodies against 95F-myosin disrupts invagination of the metaphase furrows that form during syncytial development (Mermall and Miller, 1995).

Septin localization at metaphase requires invaginated membranes

Anillin and peanut (a *Drosophila* septin) are conserved components of the metaphase and cytokinesis furrows and show a similar pattern of localization, both localizing early during the process of furrow formation (Miller et al., 1989; Neufeld and Rubin, 1994; Fares et al., 1995; Field and Alberts, 1995; Rothwell et al., 1998). Mutational analysis of *nuf*-derived embryos indicates that recruitment of these proteins to the developing furrows occurs independently of actin. Once the furrows are formed at metaphase, however, peanut and anillin are not maintained in regions where actin is not localized and furrow formation has failed (Rothwell et al., 1998). These results indicate that stable furrow localization of these proteins either requires actin or normally invaginated membranes.

Analysis of *dah*-derived embryos in which actin localization occurs normally but membrane invagination fails helps distinguish between these alternatives. In *dah*-derived embryos, prophase occurs relatively normally but membrane invagination during metaphase fails (Fig. 2). Actin, peanut and anillin localize normally during prophase in *dah*-derived embryos. However, during metaphase, peanut localization specifically fails while actin and anillin remain localized in the un-invaginated furrows. One interpretation of these results is that the septins require intact plasma membrane for stable localization. This interpretation is supported by co-localization and biochemical studies indicating that septins are intimately associated with membrane (Neufeld and Rubin, 1994; Fares et al., 1995; Caltagarone et al., 1998). Anillin, on the other hand, has a more diverse localization pattern in that it cycles between the nucleus and the cortex (Field and Alberts, 1995).

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