# Maternal Effect Mutations of the *sponge* Locus Affect Actin Cytoskeletal Rearrangements in *Drosophila melanogaster* Embryos

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***Abstract.*** In the syncytial blastoderm stage of *Drosophila* embryogenesis, dome-shaped actin "caps" are observed above the interphase nuclei. During mitosis, this actin rearranges to participate in the formation of pseudocleavage furrows, transient membranous invaginations between dividing nuclei. Embryos laid by homozygous *sponge* mothers lack these characteristic actin structures, but retain other actin associated structures and processes. Our results indicate that the *sponge* product is specifically required for the formation of actin caps and metaphase furrows. The specificity of the *sponge* phenotype permits dissection of both the process of actin cap formation and the functions of actin caps and metaphase furrows. Our data demonstrate that the distribution of actin binding protein 13D2 is unaffected in *sponge* embryos and suggest that 13D2 is upstream of actin in cortical cap assembly. Although actin caps and metaphase furrows have been implicated in maintaining the fidelity of nuclear division and the positions of nuclei within the cortex, our observations indicate that these structures are dispensible during the early syncytial blastoderm cell cycles. A later requirement for actin metaphase furrows in preventing the nucleation of mitotic spindles between inappropriate centrosomes is observed. Furthermore, the formation of actin caps and metaphase furrows is not a prerequisite for the formation of the hexagonal array of actin instrumental in the conversion of the syncytial embryo into a cellular blastoderm.

THE early embryo of *Drosophila melanogaster* is unusual in that it is a syncytium: 13 nearly synchronous nuclear divisions precede the subdivision of the embryo into individual cells. During the earliest nuclear cycles, the nuclei are positioned within the central core of the embryo. Most nuclei migrate towards the cortex during cycles 8 and 9. By interphase of cycle 10, these nuclei form a monolayer just below the plasma membrane, and the embryo is now called a syncytial blastoderm. The nuclei undergo four rounds of division while localized within this cortical cytoplasm. Then, in early cycle 14, the invagination of membranes between the cortical nuclei converts the syncytial blastoderm into a cellular blastoderm. Mitotic synchrony is lost after cellularization, and the embryo begins gastrulation immediately (Fullilove and Jacobson, 1971; Zalokar and Erk, 1976; Foe and Alberts, 1983).

During the syncytial blastoderm stage, the cytoskeleton is thought to be important in maintaining cytoplasmic organization. Throughout development, nuclear division is mediated by centrosomes and microtubules. Before nuclear migration, the majority of filamentous actin forms a thin layer uniformly underlying the plasma membrane (see Fig. 1) (Warn et al., 1984; Karr and Alberts, 1986; Hatanaka and Okada, 1991) and actin is not observed in association with the centrally located nuclei. When the interphase nuclei and their centrosomes reach the cortex, the even actin layer is rearranged to form dome-shaped actin "caps" above the nuclei (see Fig. 2). During each syncytial blastoderm mitosis, the centrosomes duplicate, separate, and nucleate the formation of mitotic spindles (Stafstrom and Staehelin, 1984; Warn and Warn, 1986; Karr and Alberts, 1986; Warn et al., 1987; Kellogg et al., 1988). Coordinated with these changes, filamentous actin is also reorganized; during the mitosis of cycle 10, the actin cap enlarges to cover the entire region above the mitotic spindle, but actin is concentrated primarily above the two centrosomes, forming crescent-shaped "half caps" (Karr and Alberts, 1986). The actin rearrangements accompanying mitosis in cycles 11, 12, and 13 are somewhat different from cycle 10 (probably because of the higher nuclear density in these older embryos): during prophase, filamentous actin becomes more concentrated towards the nuclear periphery, in the region where transient surface membrane invaginations are beginning to form; by metaphase, actin underlies the entire plasma membrane, thereby coating the invaginated membranes which physically separate adjacent mitotic spindles (see Fig. 3); between late anaphase and the next interphase, the furrows disappear and actin caps are reformed (Warn et al., 1984; Karr and Alberts, 1986; Kellogg et al., 1988). After the thirteenth mitosis, actin forms a membrane-associated array of interconnected hexagons that mediates cellularization by invaginating between adjacent nuclei (Warn and Magrath, 1983).

While the morphology of actin structures in wild-type syncytial blastoderm embryos has been well described, the mechanisms responsible for their formation and modulation during the cell cycle are unclear. Limited data indicate that centrosomes may have a role in orchestrating actin rearrangements: centrosomes migrate to the periphery along with the somatic nuclei, and their arrival results in the conversion of actin from a uniform layer into individual caps. The initial cap is positioned above the centrosome, and the cycling of actin structures during cycle 10 most closely parallels the movements of the centrosomes (Karr and Alberts, 1986). The migration of centrosomes to the cortex without nuclei results from certain maternal effect mutations (*gnu*, Freeman et al., 1986; and *fs(1)Ya*, Lin and Wolfner, 1991) or from the treatment of premigratory wild-type embryos with either aphidicolin (Raff and Glover, 1989) or UV irradiation (Yasuda et al., 1991). These free centrosomes are sufficient for the formation of actin caps (Raff and Glover, 1989). When the free centrosomes divide and separate, the actin is correspondingly altered (Yasuda et al., 1991). In addition, syncytial blastoderm embryos exhibiting abnormal microtubule structures, as a result of the injection of antitubulin antibodies, also exhibit aberrant actin structures (Warn et al., 1987). The manner in which centrosomes may communicate with actin filaments is not understood.

What functions do actin caps and metaphase furrows serve? The treatment of embryos with cytochalasin B, a compound that inhibits actin polymerization, has provided some hints as to the function of these actin structures. When exposed to cytochalasin B, syncytial blastoderm embryos exhibit a number of defects: chromosomes from neighboring nuclei segregate to a common pole, generating nuclei with abnormal chromosome complements and morphology, and nuclei are observed in the basal cortex, the cortical region which is farthest from the plasma membrane and normally devoid of nuclei (Zalokar and Erk, 1976; Edgar et al., 1987). Since actin caps and metaphase furrows are the only obvious actin structures in the syncytial blastoderm embryo, these data may suggest a role for the actin metaphase furrows in physically separating adjacent mitotic apparati and a role for actin caps in keeping nuclei in close proximity to the plasma membrane.

In all cells, actin is associated with a large number of other proteins; these various actin binding proteins are thought to mediate the formation and rearrangement of actin structures (Weeds, 1982). A collection of actin binding proteins from early *Drosophila* embryos was recently obtained by actin affinity chromatography (Miller et al., 1985; Miller and Alberts, 1989). Immunolocalization of these actin binding proteins has revealed that the actin-based cytoskeleton of syncytial blastoderm embryos is a complex network of interacting proteins with many levels of organization (Miller et al., 1989). While *Drosophila* actin binding proteins can be grouped into classes whose members associate with subsets of cortical actin structures, the functions of these proteins are largely undefined. Analysis of the effects of developmental perturbations on actin binding proteins should clarify their roles in normal embryogenesis.

Some insight into the function of the actin structures and the mechanism of their rearrangement can be gained from examination of the phenotype of mutant embryos that exhibit defects during the syncytial blastoderm stages. Here we report the identification and characterization of a mutation which results in the absence of actin caps and metaphase furrows. Our analysis indicates that the primary defect in embryos derived from *sponge* mutant mothers is in the initial formation of the actin structures. The defect in actin organization leads to subsequent abnormal microtubule structures during metaphase and aberrant nuclear divisions. However, not all aspects of the cortical actin cytoskeleton are disrupted, since at least one actin binding protein is normally organized in *sponge* embryos. We present a working model for the formation of actin caps which accounts for these results. Our results suggest that actin caps are not essential for maintaining nuclei in the cortex, but that metaphase furrows are critical to prevent the formation of aberrant mitotic spindles. In addition, we have observed that embryos which lack actin caps and metaphase furrows can still form relatively normal hexagonal arrays of actin filaments that can invaginate to form cells in cycle 14. This demonstrates that the actin–myosin network which mediates cellularization forms independently of the actin caps and metaphase furrows that are present in the preceding cell cycles.

## Materials and Methods

### Stocks and Complementation Tests

Six alleles of the *sponge* locus were isolated in various screens in the laboratory of C. Nüsslein-Volhard (Max Planck Institute for Developmental Biology, Tübingen, Germany) and the locus was mapped to 3–95 (unpublished). We have examined the six alleles generated in Tübingen. The mutagenized chromosomes carrying alleles 335, 383, 805, and 842 are homozygous viable and female sterile. Alleles 145 and 242 were originally associated with recessive lethal mutations elsewhere on the chromosome. The lethal mutations were eliminated by recombination and the resulting lines carrying alleles 145 and 242 are homozygous viable and female sterile. All alleles were tested in trans to one another to determine allele strength. The embryos derived from such heteroallelic combinations were examined to see how far they progressed through embryogenesis. Embryos from mothers with any combination of alleles 145, 242, and 335 die soon after gastrulation. Other allele combinations allow more extensive embryonic development. Deficiencies spanning from 95F to 98A (kindly provided by Kathryn Anderson, University of California, Berkeley, CA) fail to uncover the *sponge* locus. Ore-R was used as our wild-type stock.

### Videotapes

Embryos were collected for 30 min from homozygous $spg^{335}$ females that had been mated to their heterozygous and/or homozygous brothers. The embryos were covered with Voltalef oil and an 18 × 18-mm cover slip and placed on Petriperm plates. The embryos were filmed on a time lapse video cassette recorder (Panasonic AG-6030) for up to 3 h. The timing of developmental events such as yolk contractions, pole bud emergence, nuclear migration, pole cell formation, and cellularization was compared with that of Ore-R embryos.

### Immunofluorescence

Embryos were collected from Ore-R stocks and from homozygous $spg^{335}$ females that had been mated to their brothers. The embryos were dechorionated with bleach for 1 min and fixed for 30 min in a mixture of heptane and 18.5% formaldehyde. If the embryos were to be stained with a tubulin antibody, 10 μl of 0.5 mM taxol solution in DMSO (Karr and Alberts, 1986) was added to the fixative, resulting in a final taxol concentration of $8.33 \times 10^{-7}$ M. The fixed embryos were manually devitellinized in PBS (Wieschaus and Sweeton, 1988). Occasionally, embryos were postfixed in 9% formaldehyde for 15 min. The embryos were washed in PBS and PBS with 0.1% Triton X-100, 4% BSA, and 0.1% sodium azide (PBT). They were incubated in PBT at room temperature for at least 1 h. Then, the embryos were incubated with the primary antibody overnight at 4°C. The embryos were washed with PBT and PBS with 0.1% Triton X-100 (PBS–Triton) and were incubated in PBS–Triton for 30 min. They were incubated with the

appropriate preabsorbed secondary antibody for ~2 h. The embryos were then washed in PBS-Triton and PBS. To visualize filamentous actin, embryos were stained for 20 min with either 0.165 μM bodipy phalloidin or 0.165 μM rhodamine phalloidin (Molecular Probes, Inc., Eugene, OR). After several washes in PBS-Triton, they were incubated for 3 min with 1 μg/ml Hoechst 33258 (Polysciences Inc., Warrington, PA), a DNA specific dye. The embryos were washed extensively in PBS-Triton and PBS before being mounted in Aquapolymount (Polysciences Inc.).

### Antibodies

Mouse monoclonal anti-β-tubulin (Amersham Corp., Arlington Heights, IL) antibody was used on embryos at a concentration of 1:250. A rabbit polyclonal antibody to the centrosomal protein DMAP 190, graciously provided by Doug Kellogg (University of California, San Francisco, CA), was diluted 1:250 before staining embryos. Antibodies to actin binding proteins were described in Miller et al. (1989). The following concentrations were used for embryo stainings: hybridoma culture supernatant containing mAbs 13D2, 13G5, and 11B5 were diluted 1:10, while polyclonal antisera were used at 1:200. Dilutions were made in PBS containing 4% BSA. The secondary antibodies used include: rhodamine goat anti-mouse IgG, rhodamine goat anti-rabbit IgG, and rhodamine goat anti-mouse IgM (from Boehringer-Mannheim Corp., Indianapolis, IN). All secondary antibodies were preabsorbed overnight at 4°C with 100 μl of fixed, devitellinized embryos. They were diluted between 1:200 and 1:500 in PBS with 1% BSA and 0.1% Triton X-100 before being used for staining embryos.

### Quantitation of Centrosomal, Mitotic, and Nuclear Defects

Fixed populations of *sponge* embryos were stained with Hoechst, phalloidin, and an antibody to either β-tubulin or a centrosomal protein. They were compared with wild-type embryos of the same nuclear cycle. Embryos were examined for potential defects in: nuclear morphology, spacing between nuclei, positioning of nuclei relative to the plasma membrane, centrosome morphology, centrosome position within the apical cytoplasm, centrosome duplication, centrosome separation, spindle formation, and chromosome separation. Cortical nuclei were considered to have a basal localization if they were farther from the surface than the nuclei of wild-type embryos. Nuclei separated by a distance less than two thirds of the average distance between nuclei were considered abnormally close; a given nucleus need only have one very close neighbor to be scored as abnormal and each nucleus was counted only once.

## Results

The *sponge* locus is represented by seven EMS-induced alleles. The original allele, isolated by Rice and Garen (1975), was known as *mat(3)6.* Six more alleles were isolated in the laboratory of Christiane Nüsslein-Volhard (unpublished observation). All alleles of *sponge* show a recessive maternal effect: females homozygous for *sponge* mutations lay morphologically normal eggs which never hatch. A consistent set of developmental defects is seen in such "*sponge* embryos." In addition, females carrying different allele combinations of *sponge* bear embryos with a similar phenotype: syncytial blastoderm embryos exhibit defects in cortical actin structures and the divisions of cortical nuclei; cellularization is incomplete, and development becomes increasingly aberrant thereafter. The severity of these defects varies from one allele to another (see Materials and Methods for details). Embryos derived from mothers carrying weak *sponge* alleles have aberrant regions interspersed with regions of normal morphology. In contrast, females with strong *sponge* alleles lay embryos with defects that are uniform and encompass the entire blastoderm. These embryos form few cells and arrest after a feeble attempt at gastrulation. Since there is no deficiency which uncovers the *sponge* locus, it is not known whether any of the *sponge* alleles is a null. Our analysis has concentrated on the strong allele 335.

### Actin Caps Fail to Form in Cycle 10 in sponge Embryos

Eggs produced by homozygous *sponge* mothers are successfully fertilized and the resulting embryos develop normally during the initial cleavage stages. The nuclear divisions occur with the same frequency as in the wild-type and are accompanied by yolk contractions. Filamentous actin, as visualized with phalloidin, is present as a punctate cortical monolayer in both *sponge* and wild-type embryos (Fig. 1). Nuclear migration, an actin-dependent process (Zalokar and Erk, 1976; Hatanaka and Okada, 1991), occurs normally, first along the anterior–posterior axis of the embryo and then towards the embryo's periphery. Pole buds appear in the posterior of the embryo at the appropriate time and are pinched off into cells, presumably by contractile rings of actin and myosin (Warn et al., 1985).

In cycle 10, the somatic nuclei of *sponge* embryos reach the cortical cytoplasm in near synchrony and distribute themselves evenly throughout the cortex (Fig. 2, *G* and *H*). As in wild-type (Fig. 2, *C* and *D*), each interphase nucleus is spherical, has decondensed chromosomes, and is associated with a single centrosome. The vast majority of the nuclei occupy a single plane just a few micrometers under the plasma membrane. The tubulin-based cytoskeleton is normal in cycle 10 *sponge* embryos: microtubule arrays emanate from centrosomes, and the distribution of the centrosomal protein DMAP 190 (identified by Ab S1-4 in Kellogg et al., 1989 and as Rb188 in Whitfield et al., 1988) is indistinguishable from wild-type. As the nuclei proceed through cycle 10, the centrosomes duplicate normally and separate to the same extent as in wild-type (see Fig. 4, *E–H* for cycle 11 embryo demonstrating this point). The mitotic spindles organized in *sponge* embryos appear identical to wild-type, and the 10th nuclear division proceeds in an orderly and precise manner (Fig. 4, *A–D*).

However, rare defects were observed in cycle 10 *sponge* embryos. For example, ~6.0% (65/1175) of the interphase *sponge* nuclei have slightly more basal positions, a feature rarely observed in wild type (9/1132; $P < 0.005$). In addition, the orientation of centrosome migration was not always precise: in 4.1% (46/1175) of the nuclei, one of the duplicated centrosomes migrated below the nucleus. Such subnuclear centrosomes were only seen in 0.4% (4/1132; $P < 0.005$) of wild-type nuclei. These nuclei divide completely, but the axis of division is not parallel to the embryo's surface. Because these defects are rare and occur at the same time as the more prevalent defects in the actin cytoskeleton (see next paragraph), it is possible that they represent the first consequences of the actin abnormalities.

In contrast to the near wild-type appearance of the nuclei and microtubule-based structures, the actin-based cytoskeleton is drastically altered in cycle 10 *sponge* embryos. Actin caps do not form upon the completion of nuclear migration (Fig. 2, *E–H*), and the actin rearrangements that normally accompany mitosis in cycle 10 do not occur (data not shown). These defects in the formation of actin structures persist throughout the syncytial blastoderm stage. No actin caps are ever present in interphase embryos, and metaphase furrows fail to form during mitosis (Fig. 3, *E–H*). In addition, the plasma membrane fails to form bulges above the interphase nuclei and to transiently invaginate between mitotic nuclei (data not shown).

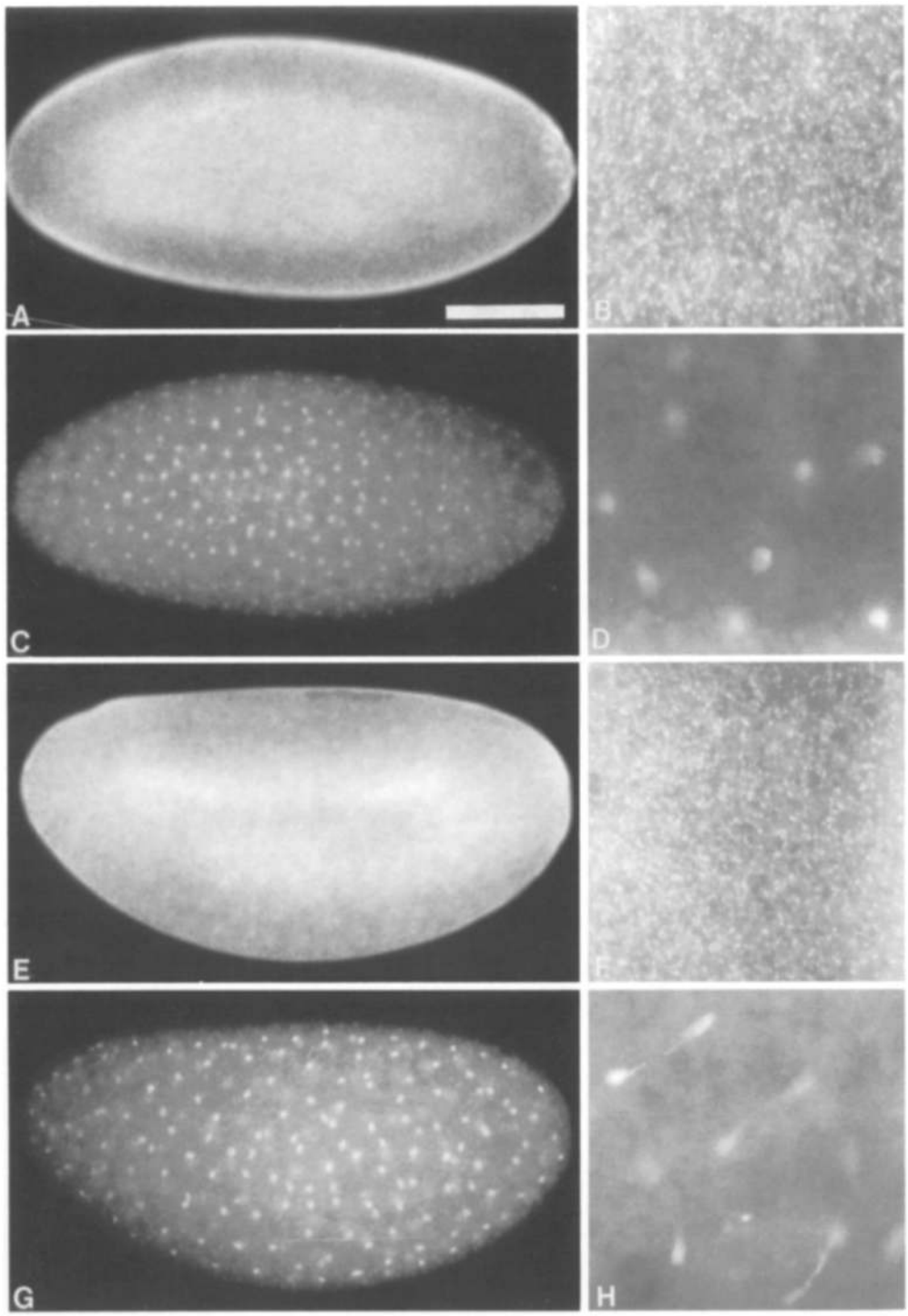



*Figure 1. sponge* embryos develop normally through the beginning of cycle 10. Wild-type embryos (*A–D*) and *sponge* embryos (*E–H*) were double labeled with rhodamine phalloidin, to visualize filamentous actin, and Hoechst 33258, to stain the nuclei; whole embryos are shown on the left and higher magnification surface views of similar stage embryos are shown on the right. In both wild-type and *sponge* preblastoderm embryos, actin (*A* and *E*) is present as a continuous monolayer underlying the entire plasma membrane. A closer examination of the actin monolayer (*B* and *F*) reveals its punctate appearance. The nuclei (*C*, *D*, *G*, and *H*) of both wild-type and *sponge* embryos divide synchronously and migrate in unison towards the embryo's periphery. Bars: (*A*, *C*, *E*, and *G*) 30 μm; (*B*, *D*, *F*, and *H*) 5 μm.

Despite their lack of actin caps and metaphase furrows, syncytial blastoderm *sponge* embryos do contain arrays of actin. Phalloidin staining (Fig. 2, *E* and *F*) reveals that filamentous actin is present as a cortical layer interrupted by zones of actin depletion, areas above each nucleus containing substantially reduced amounts of actin. The actin in the remainder of the cortical cytoplasm resembles that seen in embryos before nuclear migration. The actin-poor regions arise when nuclei appear in the cortex; they lie above the interphase nuclei, with their positions and dimensions corresponding exactly to those of the nuclei (Fig. 2, *E–H*). As the nuclei undergo mitosis, the zones of actin depletion enlarge (Fig. 3, *E–H*) and attain a maximum size during anaphase (see Fig. 8 *B*). There is a correlation between the mitotic spindle of each nucleus and the zone of actin depletion; little actin is present in the region above the mitotic spindle, and the border of the zone of actin depletion coincides with edges of the microtubule network (data not shown). Yet, the distributions of actin and tubulin are not exact inverses of one another during interphase or prophase (data not shown). It is tempting to speculate that microtubule arrays might cause zones of actin depletion by excluding actin. However, the

data only suggest a direct physical exclusion of actin by microtubules at certain times in the cell cycle.

### *Older* sponge *Embryos Exhibit Major Flaws in Nuclear Morphology, Spacing, and Division*

Disruption of actin filaments by cytochalasin has been reported to cause gross nuclear defects in syncytial blastoderm embryos: chromosomes from different mitotic nuclei "collide" during anaphase; nuclei with abnormal chromosome complements and morphology are observed; and nuclei become displaced from the apical cytoplasm (Zalokar and Erk, 1976; Edgar et al., 1987). While these data indicate that actin-dependent structures are needed to preserve the integrity of nuclear divisions within the syncytium and to maintain the somatic nuclei in a cortical monolayer, the experiments do not address which structures perform these tasks. Since *sponge* embryos contain some actin structures but specifically lack actin caps and metaphase furrows, we examined the phenotype of *sponge* embryos in attempt to identify processes requiring actin caps and/or metaphase furrows.

As described earlier, actin caps and metaphase furrows never form in embryos laid by homozygous *sponge* mothers. Thus, defects arising from the absence of these structures might be expected to appear soon after the beginning of cycle 10, when actin caps are normally assembled. However, nuclear position and division during the 10th cycle are largely unaffected (see previous section for details). *sponge* embryos in interphase of cycle 11 also appear quite normal. All of the nuclei exhibit normal morphology and 98.5% (2637/2675) retain their positions in the apical cytoplasm. Only a slight nuclear defect is noticeable: 6% of the nuclei (149/2675) are in closer proximity to an adjacent nucleus than is ever seen in a wild-type cycle 11 embryo (0/1785; $P < 0.005$). As the cell cycle proceeds, centrosome duplication and separation occur as in wild-type (Fig. 4, *E–H*). But when mitotic spindles form during metaphase of cycle 11, they are often aberrant: spindles are nucleated between centrosomes from different nuclei (a cycle 13 embryo with aberrant microtubule arrays is shown in Fig. 4, *J* and *L*). These abnormal spindles are most frequently formed between nuclei that are unusually close to one another. Although the abnormal spindles are capable of separating chromosomes, the fidelity of the division is compromised.

*sponge* embryos become progressively more aberrant as they proceed through cycles 12 and 13. Many nuclei have altered morphologies which are consistent with the preceding mitotic defect: bi-lobed and tri-lobed nuclei containing many centrosomes are visible as are small, dense nuclei that lack centrosomes or have a single, nondividing centrosome. Both classes of nuclei fail to divide (see *arrowheads* in Fig. 4, *J* and *L*) and become displaced from the apical cytoplasm. The spacing between cortical nuclei is irregular in the late syncytial blastoderm *sponge* embryo as well; nuclei are often clustered together in certain regions whereas other areas of the same embryo have many fewer nuclei. Among those nuclei that continue to divide, abnormal spindles are a common occurrence (Fig. 4, *J* and *L*). These defects, observed in *sponge* embryos from cycles 12 and 13, correlate quite well with the reported results of cytochalasin treatment.

### sponge *Embryos Partially Cellularize during Cycle 14*

After the fourth syncytial blastoderm cleavage (i.e., interphase of cycle 14), actin caps are briefly reformed in wild-type embryos. Although the nuclei remain in a prolonged interphase, the actin caps soon enlarge and adjacent caps touch at their bases. At the level of the cap bases, actin forms a roughly hexagonal network. The network is refined during nuclear elongation, and it changes in appearance from a meshwork of actin to discrete lines of actin filaments (Simpson and Wieschaus, 1990). This mature hexagonal array of actin (Fig. 5, *A–D*) mediates the subsequent subdivision of the embryo into individual cells; along with the plasma membrane, it invaginates between adjacent nuclei and pinches off the new cells at their bases (Warn and Magrath, 1983).

By cycle 14, most *sponge* embryos are quite abnormal. The majority of nuclei have become displaced from the apical cytoplasm, and actin above these regions forms a continuous monolayer, reminiscent of that seen in preblastoderm embryos. The nuclei that remain closely apposed to the plasma membrane are usually relatively isolated and have zones of actin depletion associated with them. Wherever a number of morphologically normal nuclei are clustered, a hexagonal array of actin filaments forms around them (Fig. 5, *E* and *F*). While these groups of spherical, evenly spaced nuclei may occur throughout the cortex, they are most commonly present at the embryo's poles. Unlike the nuclei of wild-type embryos, which elongate along their apical–basal axis in early cycle 14 (Fullilove and Jacobson, 1971), the "normal" nuclei in *sponge* embryos remain spherical.

The hexagonal actin network formed in cycle 14 *sponge* embryos differs from that of wild-type in two major respects. First, *sponge* embryos contain thin sheets of actin filaments which cover the area between the edges of the nuclei and the hexagonal lines of actin (Fig. 5, *E* and *F*). It appears as if the hexagonal actin array is superimposed on the earlier pattern of a cortical layer of actin interrupted by zones of actin depletion. Secondly, the size of the individual units that make up the hexagonal array is larger in *sponge* embryos. This is due to the lower density, and corresponding larger size, of cortical nuclei in cycle 14 *sponge* embryos. Despite these morphological abnormalities and the fact that it does not encompass the entire blastoderm, the actin network is cellularization competent; *sponge* embryos contain invaginated actin arrays and pinched off cells (Fig. 5, *G* and *H*). The cells which do form are usually much shallower than those seen in wild-type.

### *Actin Binding Proteins in* sponge *Embryos*

While syncytial blastoderm *sponge* embryos lack actin caps and metaphase furrows, these embryos contain filamentous actin and form ordered actin structures before nuclear migration and during cellularization. The *sponge* mutation seems to primarily affect the organization of actin structures during the syncytial blastoderm stage. To determine whether all aspects of cortical actin-based organization are disrupted by the *sponge* mutation, we examined the distribution of several actin-associated proteins in syncytial blastoderm *sponge* embryos. These putative actin binding proteins were biochemi-

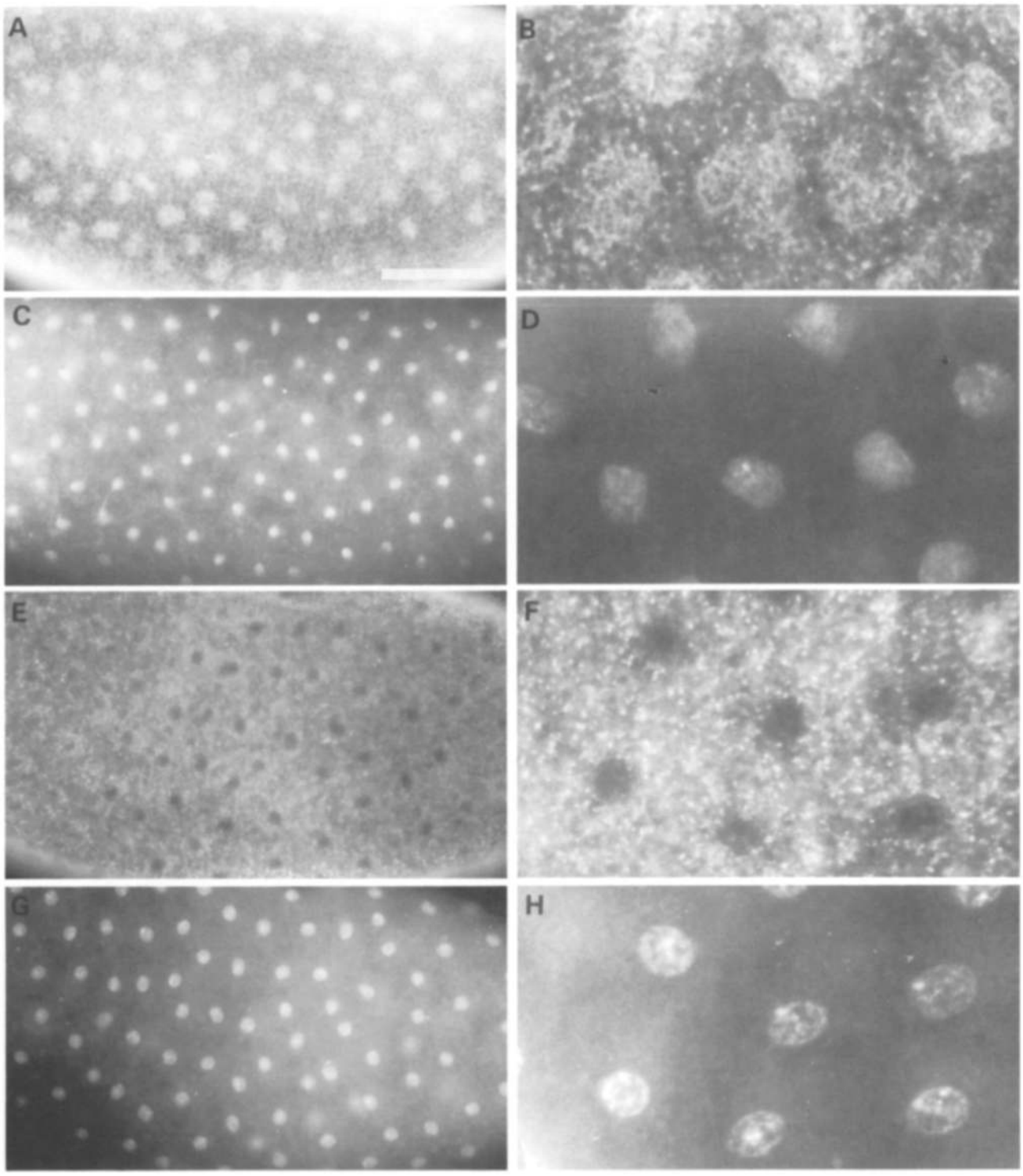



*Figure 2. sponge* nuclei lack actin caps. Surface views of double-labeled wild-type (*A–D*) and *sponge* (*E–H*) embryos are shown in lower power on the left and higher power on the right. An actin cap (*A* and *B*) is organized above each cortical nucleus (*C* and *D*) in a wild-type syncytial blastoderm embryo. While the nuclei (*G* and *H*) of *sponge* embryos arrive in the cortex during early cycle 10, actin filaments (*E* and *F*) fail to form caps above the nuclei. Instead, *sponge* embryos contain filamentous actin (*E* and *F*) in the form of a monolayer interrupted by zones of actin depletion which correspond to the positions and sizes of the nuclei (*G* and *H*). The punctate appearance of the interrupted actin monolayer is reminiscent of the premigratory actin monolayer (see Fig. 1). Bars: (*A*, *C*, *E*, and *G*) 15 μm; (*B*, *D*, *F*, and *H*) 4.25 μm.

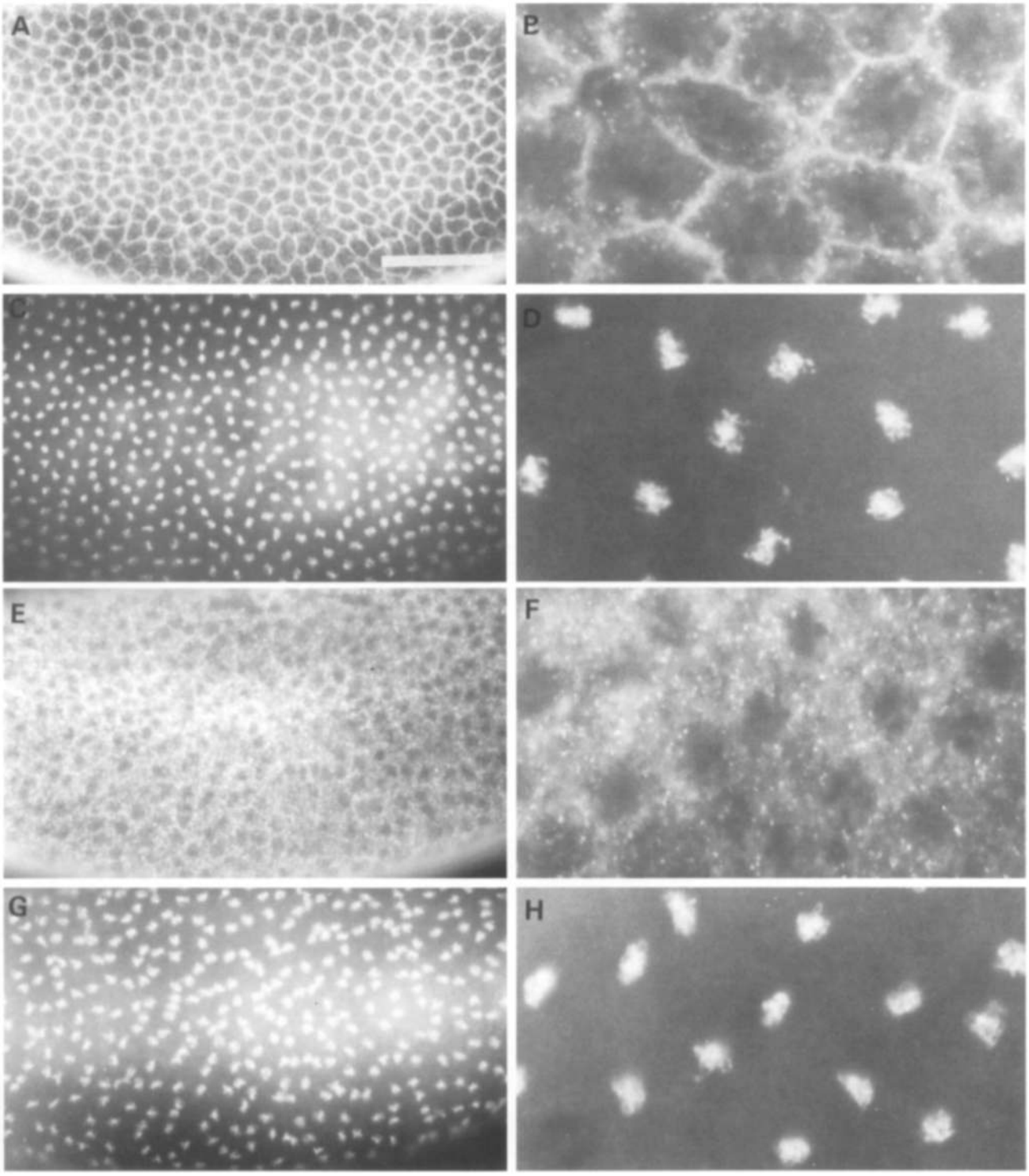
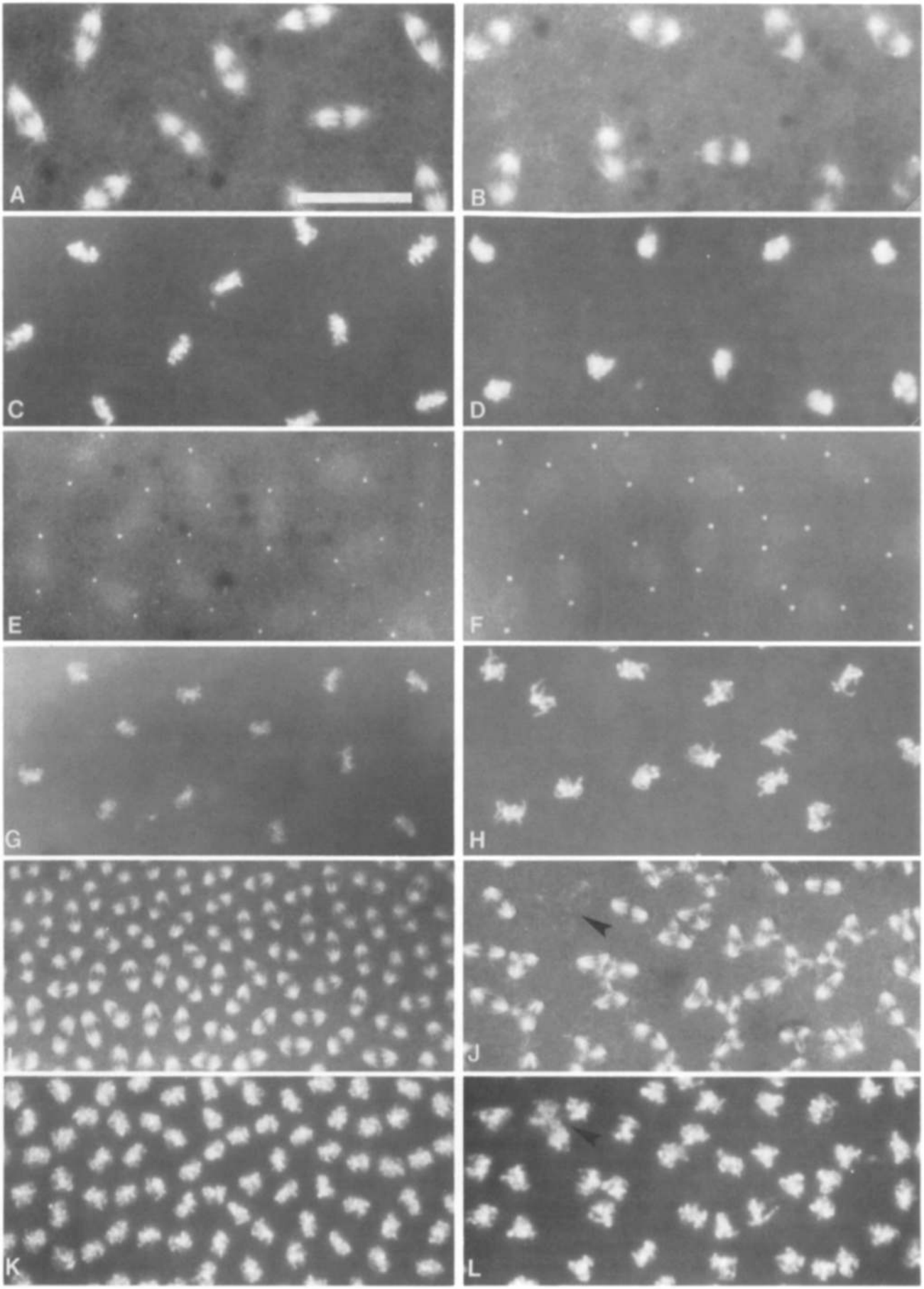



*Figure 3. sponge* embryos lack metaphase furrows. Surface views of double-labeled wild-type (*A–D*) and *sponge* (*E–H*) embryos are shown in lower power on the left and higher power on the right. As the nuclei (*C* and *D*) of wild-type syncytial blastoderm embryos divide, actin structures (*A* and *B*) are rearranged. In metaphase, actin is present in transient furrows which are perpendicular to the embryo's surface and physically separate adjacent dividing nuclei. The nuclei (*G* and *H*) of *sponge* embryos divide in synchrony, but actin filaments (*E* and *F*) never form metaphase furrows. Actin remains as a punctate monolayer parallel to the embryo's surface; the zones of actin depletion enlarge slightly as the nuclei divide. Bars: (*A*, *C*, *E*, and *G*) 15 μm; (*B*, *D*, *F*, and *H*) 4.25 μm.



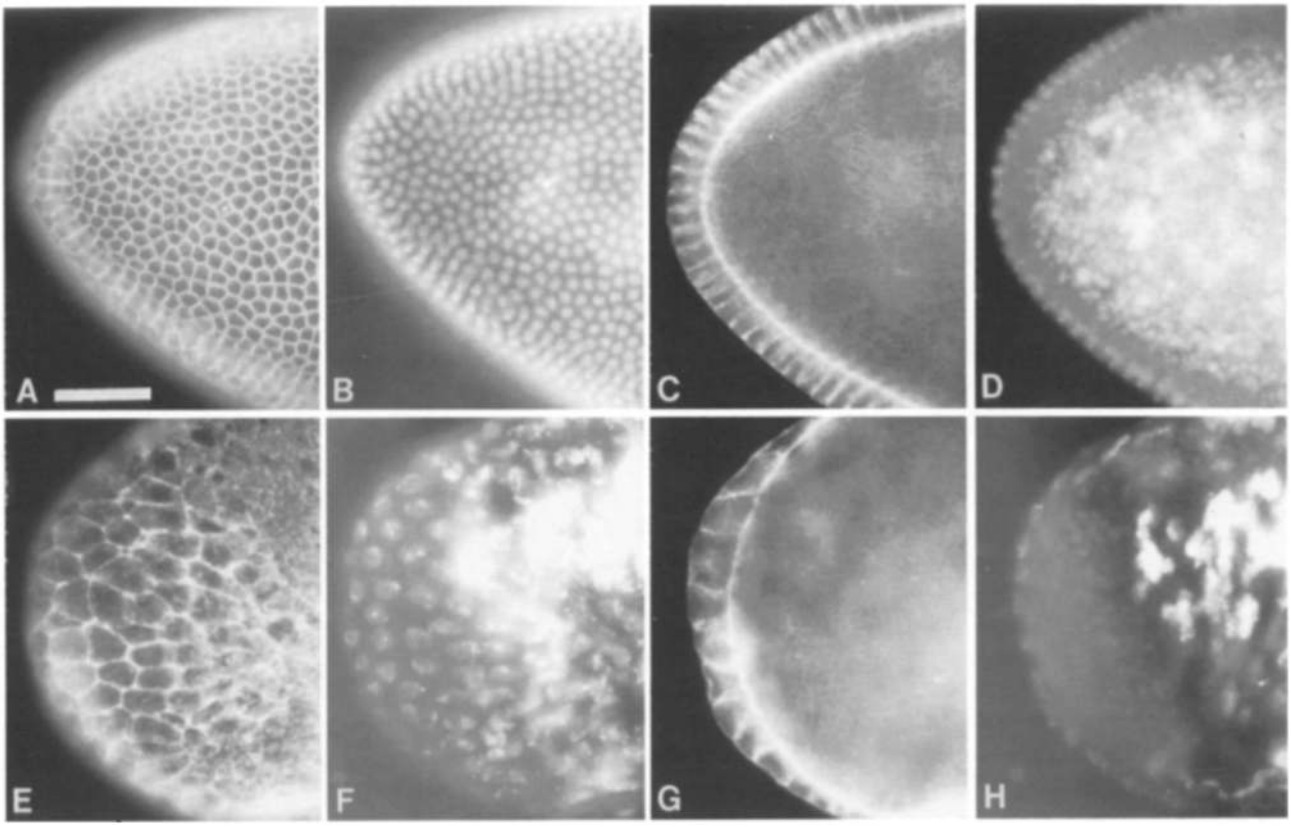
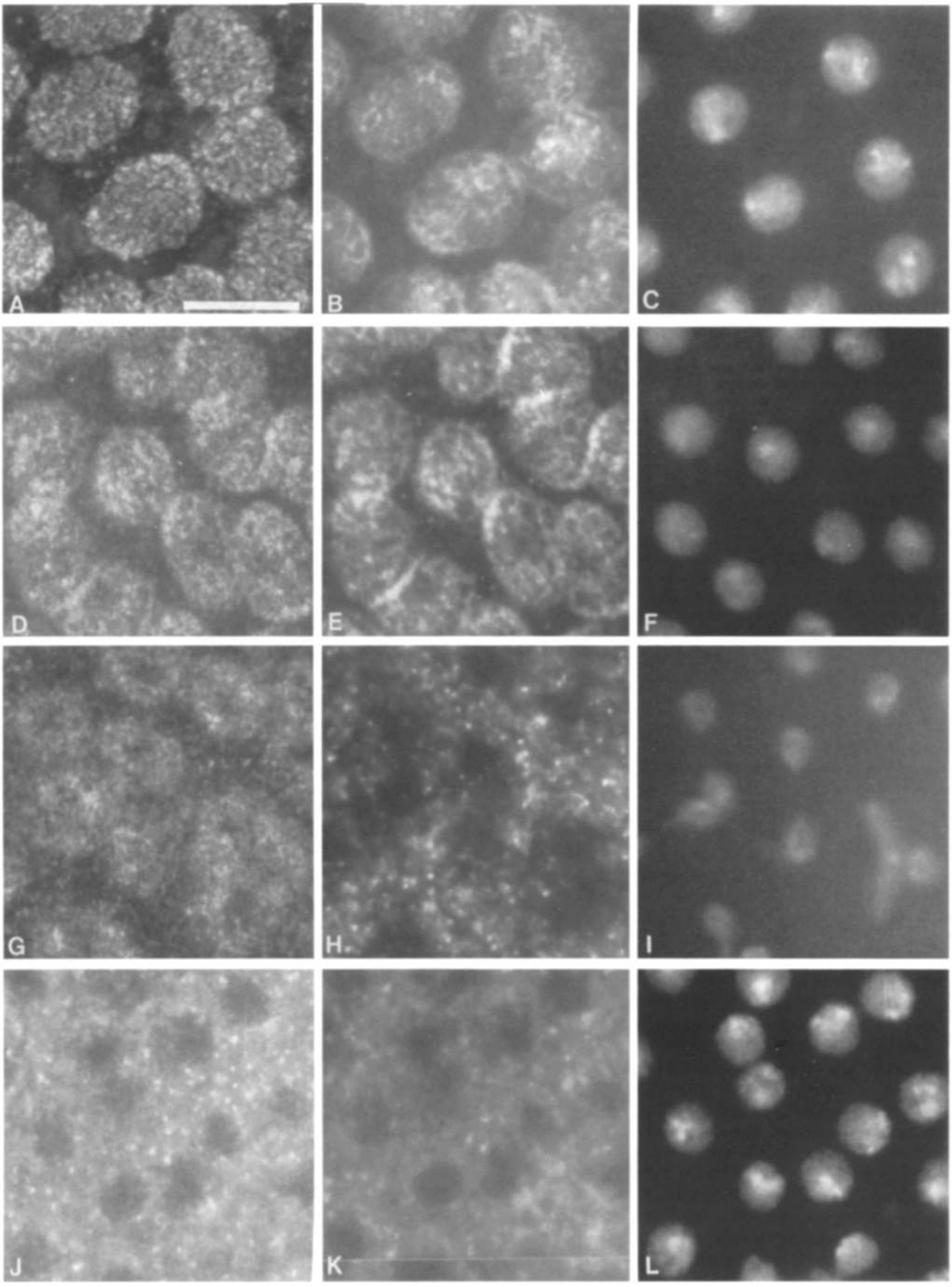



*Figure 5. sponge* embryos partially cellularize. Wild-type embryos (*A–D*) and *sponge* embryos (*E–H*) were double-labeled with rhodamine phalloidin and Hoechst. Surface views of embryos are shown on the left, and optical cross sections of similar stage embryos are shown on the right. During cycle 14, wild-type embryos form an actin network (*A*) which covers the entire embryo, enclosing each nucleus (*B*) in a hexagon of actin filaments. The actin network (*C*), together with the plasma membrane, invaginates into the embryo and subdivides the nuclei (*D*) into individual cells. In cycle 14 *sponge* embryos, actin filaments (*E*) form patches of interconnected hexagons enclosing morphologically normal nuclei (*F*). The actin network does not encompass the entire embryo and varies from wild-type in terms of hexagon size and appearance. The hexagonal array of actin (*G*) in *sponge* embryos invaginates, forming shallow cells containing single nuclei (*H*). The intense fluorescence seen in the central regions of Hoechst-stained embryos (*B, D, F,* and *H*) is due to the presence of yolk nuclei and autofluorescent yolk vesicles in the embryo's interior. Bar, 10 μm.

cally isolated from syncytial embryos, and their distributions in wild-type *Drosophila* embryos have been described (Miller et al., 1989). The proteins can be grouped into three classes that reflect their predominant association with actin structures present at metaphase (i.e., furrows), at interphase (i.e., caps) or both (actin-like); yet each protein, when examined throughout the cell cycle, has a unique distribution. Since these proteins apparently associate with subsets of actin structures at different stages of the cell cycle, their distributions in *sponge* embryos might reveal levels of organization of the actin cytoskeleton not detectable with simple phalloidin staining.

We examined the distribution of eight different actin binding proteins in *sponge* embryos, using mAbs 13D2, 13G5, and 11B2 (Miller et al., 1985) and polyclonal antisera 2, 4, 8, 12, and 16 (Miller et al., 1989). The staining patterns of these antibodies represent all three classes of actin binding proteins. Staining at approximately normal intensity was observed with all the antibodies used, indicating that all of the antigens are present. Consistent with the previously described actin abnormalities in *sponge* embryos, in all cases except one, the structures normally labeled by these antibodies were not observed (see below). The one exception was the staining pattern of the mAb 13D2, which appeared relatively normal. In wild-type embryos, the 13D2 antigen (Fig. 6 *A*) is concentrated in caps above syncytial nuclei (Fig. 6

*Figure 4.* Nuclear division occurs normally in young syncytial blastoderm *sponge* embryos, but becomes aberrant in older *sponge* embryos. Wild-type embryos (left) and *sponge* embryos (right) were double-labeled as follows: with a β-tubulin antibody and the nuclear stain, Hoechst 33258, in panels *A–D* and panels *I–L*; and with DMAP 190 antibody and Hoechst in panels *E–H*. During cycle 10 in both wild-type (*A* and *C*) and *sponge* (*B* and *D*) embryos, condensed metaphase chromosomes (*C* and *D*) are surrounded by discrete, well-formed mitotic spindles (*A* and *B*). As demonstrated in cycle 11 embryos (*E–H*), all dividing nuclei (*G* and *H*) in both wild-type (*E* and *G*) and *sponge* (*G* and *H*) embryos have fully separated centrosomes (*E* and *F*). Some of the nuclei in the *sponge* embryo are in closer proximity to their neighbors than is ever seen in wild-type. While cycle 13 wild-type embryos (*I* and *K*) have evenly spaced cortical nuclei (*K*) with individual mitotic spindles (*I*), cycle 13 *sponge* embryos (*J* and *L*) have clustered nuclei (*L*) and mitotic spindles (*J*) connecting centrosomes from several nuclei. As shown by the arrowhead, some *sponge* nuclei (*L*) have abnormal morphologies and lack mitotic spindles (*J*). Bar, 5 μm.



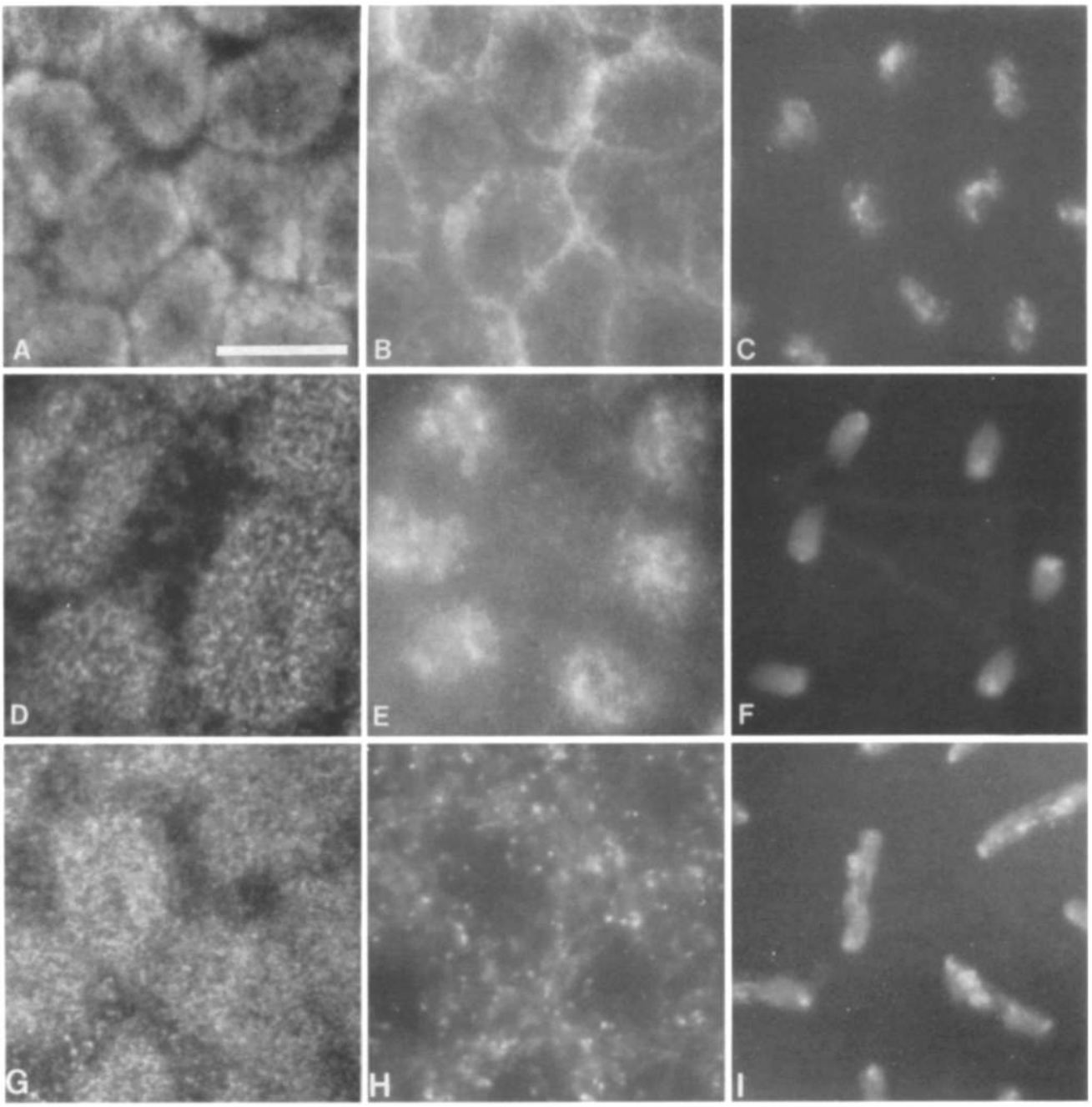



*Figure 7.* Actin binding protein 13D2 forms cell cycle regulated structures in *sponge* embryos. Wild-type (*A–F*) and *sponge* (*G–I*) embryos were triple-labeled with 13D2 antibody (*A*, *D*, and *G*), fluorescein phalloidin (*B*, *E*, and *H*), and Hoechst (*C*, *F*, and *I*). During mitosis in wild-type embryos, 13D2 caps (*A* and *D*) enlarge and elongate in a manner coordinated with spindle rearrangements (as reflected by chromosome position) (*C* and *F*). During cycles 11, 12, and 13, 13D2 (*A*) remains at the embryo's surface and is excluded from the furrows where actin (*B*) accumulates. No metaphase furrows are formed in cycle 10 (*D–F*) and the enlarged 13D2 caps (*D*) cover the entire region of the actin half-caps (*E*). As in wild-type, 13D2 structures (*G*) enlarge during mitosis in *sponge* embryos. 13D2 expansion is coordinated with spindle rearrangement (as reflected by chromosome position) (*I*) and takes place despite the absence of normal actin structures (*H*) and the lack of pseudocleavage furrows. The enlarged 13D2 caps found in mitotic *sponge* embryos throughout the syncytial blastoderm cycles resemble the 13D2 caps seen in wild-type embryos during the 10th division. The embryos depicted in panels *A–C* and *D–F* are in telophase while the embryo shown in panels *G–I* is in anaphase. Bar, 5 μm.

*Figure 6.* Actin binding protein 13D2 forms caps in interphase *sponge* embryos. Wild-type (*A–F*) and *sponge* (*G–L*) embryos were triple stained to reveal either 13D2 (*A* and *G*) or 13G5 (*D* and *J*); actin (*B*, *E*, *H*, and *K*); and DNA (*C*, *F*, *I*, and *L*). In syncytial blastoderm wild-type embryos during interphase, actin binding proteins 13D2 (*A*) and 13G5 (*D*) are present in caps that colocalize with actin (*B* and *E*) and are centered above the cortical nuclei (*C* and *F*). Interphase *sponge* embryos have 13D2 caps (*G*) above the nuclei (*I* and *L*), but lack 13G5 caps (*J*) and actin caps (*H* and *K*). Instead, 13G5 and actin are present in punctate structures in the cortical cytoplasm that are interrupted by zones of depletion above the nuclei. Bar, 5 μm.

C) during interphase. The caps are positioned identically to the actin caps (Fig. 6 *B*) and are the same size and shape. In *sponge* embryos, similar 13D2 caps (Fig. 6 *G*) were observed associated with nuclei (Fig. 6 *I*) in the cortex in the absence of actin caps (Fig. 6 *H*). These caps are coincident with the zones of actin depletion, but are larger, overlapping the regions of the cortex that contain punctate actin arrays. In both wild-type and *sponge* embryos, some faint staining is observed with 13D2 antibody in regions of the cortex outside of caps. In *sponge* embryos, a 13D2 cap is always positioned directly over each cortical nucleus, as it would be in wild-type embryos. In contrast, all of the other antibodies we tested had staining patterns that were quite abnormal. We show the pattern of the mAb 13G5 as an example. In wild-type embryos, the 13G5 antigen (Fig. 6 *D*), like 13D2, colocalizes with actin (Fig. 6 *E*) in caps during interphase; however, this antibody does not stain caps in *sponge* embryos. Instead, the 13G5 antigen (Fig. 6 *J*) is present in *sponge* embryos as a punctate cortical layer interrupted by zones of depletion; its distribution is indistinguishable from that of actin (Fig. 6 *K*).

As with actin, the distribution of these antigens changes with the cell cycle in both wild-type and *sponge* embryos. The 13D2 antigen normally colocalizes with actin only during interphase. In wild-type embryos during mitosis in cycle 10, actin caps (Fig. 7 *E*) separate into two "half caps" above each spindle pole. The corresponding 13D2 caps (Fig. 7 *D*) are larger than the actin half caps. During the remaining syncytial blastoderm mitoses, actin rearranges into furrows (Fig. 7 *B*) and coats the transiently invaginating plasma membrane. However, 13D2 does not rearrange into furrows during mitosis. It remains in a cap-like structure (Fig. 7 *A*) that appears larger than at interphase and is only associated with the non-invaginated plasma membrane. 13D2 is more concentrated near the pseudocleavage furrows, but excluded from them (compare 13D2 caps in Fig. 7 *A* to actin furrows in Fig. 7 *B*). As in wild-type embryos during mitosis, the 13D2 caps (Fig. 7 *G*) in *sponge* embryos are aligned over the spindles and appear larger than in interphase. In *sponge* embryos, the 13D2 caps are located in the regions of actin depletion but are larger than these zones, overlapping the regions of punctate actin staining (Fig. 7 *H*) at their edges, where actin is sometimes seen to accumulate. The extent of this overlap is similar to that seen during interphase. In *sponge* embryos, the 13D2 caps present during all of the syncytial mitoses have a similar appearance to those present at nuclear cycle 10 in wild-type embryos; the regions between caps have lower levels of antigen present. In *sponge* embryos, membrane regions between the caps correspond to regions that normally invaginate in wild-type embryos; the reduced 13D2 antibody staining of these membrane regions correlates with the distribution of 13D2 in wild-type embryos. The changing distribution of 13D2 during the cell cycle suggests that in both wild-type and in *sponge* embryos 13D2 is actively arranged by cell cycle-specific cues.

In contrast to the organized distribution of 13D2 antigen during mitosis, staining with mAb 13G5 (Fig. 8 *A*) is seen throughout the cortical cytoplasm during mitosis in *sponge* embryos, except where it is physically excluded by the condensed chromosomes. While the distribution of the 13G5 antigen remains similar to that of actin (Fig. 8 *B*), actin is excluded from a larger region which 13G5 is apparently free to invade. The difference in distribution between 13G5 and actin during mitosis is interesting, since it supports the idea that although actin caps and furrows do not form, some actin-based organization remains. Clearly, some components of the cortex are being rearranged during the cell cycle and some aspects of the communication between the microtubule and actin-based cytoskeletons remain active in *sponge* embryos.

## Discussion

The phenotype of embryos from mothers that carry *sponge* mutations has yielded new insights into actin structures in the early *Drosophila* embryo. *sponge* embryos develop normally before cycle 10. A defect in the organization of actin filaments and a number of actin binding proteins becomes apparent once a syncytial blastoderm is formed: while actin and actin associated proteins are present in the embryo, they generally fail to form the proper structures. Of the actin binding proteins examined, only 13D2 formed recognizable structures. Yet, the nuclei, centrosomes, and microtubules in cycle 10 *sponge* embryos are unperturbed. Division abnormalities in the form of multipolar mitotic spindles encompassing multiple nuclei first arise during the 11th mitosis. Since pervasive nuclear and mitotic defects only appear a full cell cycle after the actin defect and probably result from that defect, we conclude that the product of the *sponge* gene functions specifically in the proper organization of actin and other actin binding proteins into cortical caps and metaphase furrows.

### *The Organization of 13D2 Caps in* sponge *Embryos and Its Implications for Wild-Type Cap Formation*

Despite the lack of normal actin structures, actin binding protein 13D2 surprisingly forms wild-type, cell cycle-dependent structures in *sponge* embryos. This result demonstrates that cortical caps of 13D2 can be formed independently of actin caps and be modified throughout the cell cycle in the absence of the corresponding actin rearrangements. While 13D2 was isolated as an actin binding protein by biochemical criteria, it does not always colocalize with actin in wild-type embryos (13D2, unlike actin, is not present in metaphase furrows) (Miller et al., 1989). In *sponge* embryos, the localizations of actin and 13D2 are quite different. Thus, 13D2 does not fit the strictest definition of an actin binding protein. The method of purification used by Miller and colleagues (Miller and Alberts, 1989) allowed for the isolation of protein complexes which bind to actin; therefore, not every protein isolated may bind directly to actin. In fact, some of the actin binding proteins isolated in this manner were shown to be part of large complexes (Miller et al., 1989). It is plausible that 13D2 is an indirect actin binding protein which is incapable of associating with actin in *sponge* embryos. If this were the case, we would speculate that the proteins which mediate the interaction between 13D2 and actin are defective in *sponge* embryos. Alternatively, the interaction between 13D2 and actin may be direct, but dependent upon a *sponge*-mediated modification.

While 13D2 is not dependent upon actin for its organization, it is possible that actin organization may be dependent upon 13D2. Since a body of evidence suggests that actin caps are formed under the influence of centrosomes (Karr and Al-

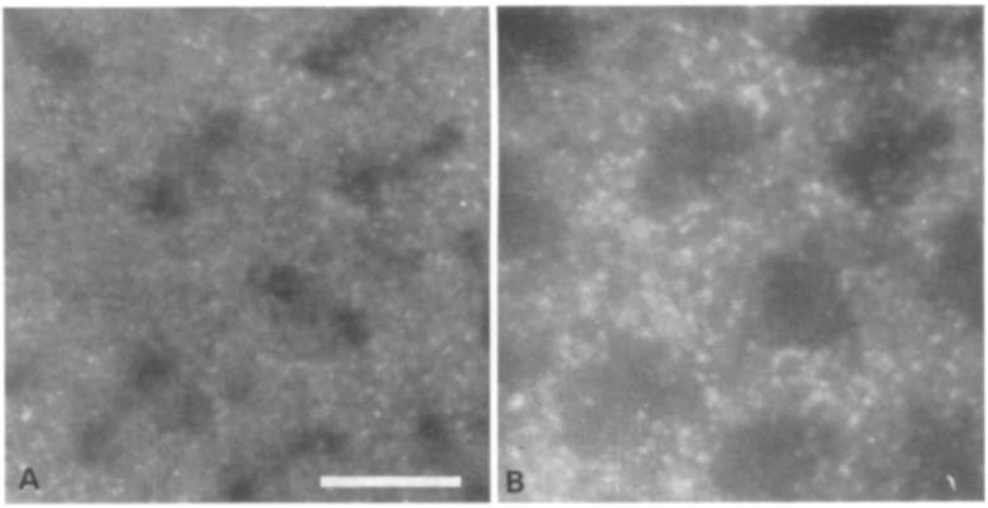



*Figure 8.* Actin binding protein 13G5 and actin are differentially localized during mitosis in *sponge* embryos. Double labeling of a *sponge* embryo with 13G5 antibody and fluorescein phalloidin reveals that 13G5 (*A*) forms a cortical monolayer interrupted only where the anaphase chromosomes are present, whereas the zone of actin (*B*) depletion includes the entire spindle region. Bar, 5 μm.

berts, 1986; Warn et al., 1987; Raff and Glover, 1989; Yasuda et al., 1991), there are likely to be proteins which mediate the interaction between the centrosomes and actin. These proteins would directly respond to the centrosomal signal and be responsible for the subsequent formation of actin caps. We favor a model in which 13D2 is such a mediator. In wild-type embryos, actin and actin binding proteins may be assembled into cortical caps using 13D2 as a framework. The 13D2 cap is present in *sponge* embryos, but the link between 13D2 and actin is severed. Thus, the presence of 13D2 caps lacking associated actin would represent an intermediate in the formation of a true cortical cap, complete with actin and all of its associated proteins. Consistent with this notion, we have observed wild-type embryos in which the 13D2 caps are more fully formed than the corresponding actin caps (K. Miller, unpublished observations). Our model predicts a functional association between 13D2 and the centrosomes. This model may be tested in part by examining the organization of 13D2 in embryos where centrosomes, but not nuclei, migrate to the periphery.

### *The Nuclear Defects in* sponge *Embryos and the Function of Actin Caps and Metaphase Furrows in Wild-Type Embryos*

When syncytial blastoderm embryos were treated with the actin disrupting drug cytochalasin B, Zalokar and Erk (1976) observed the collisions of anaphase chromosomes from neighboring nuclei, the formation of polyploid nuclei, and the presence of nuclei in the basal cortex. Edgar et al. (1987) also reported the failure of nuclei to remain in a cortical monolayer after early cycle 14 embryos were injected with cytochalasin B. These results may be interpreted to indicate that actin structures, most probably metaphase furrows, are responsible for physically separating neighboring nuclei during mitosis. In addition, it is possible that actin structures, perhaps actin caps, perform a second function of anchoring nuclei in the apical cytoplasm. Since *sponge* embryos lack actin caps and metaphase furrows but appear to have retained the ability to form actin filaments and some actin associated structures, we tested these theories by examining the defects in *sponge* embryos.

*sponge* embryos display the same defects reported for cytochalasin treated embryos. In *sponge* embryos, mitotic spindles are nucleated between centrosomes from different nuclei. This results in the segregation of chromosomes from different nuclei to a common pole and the formation of nondividing polyploid and aneuploid nuclei. Basally displaced nuclei were also observed in *sponge* embryos. Given the close correlation with the cytochalasin-dependent abnormalities, it is reasonable to assume that the nuclear defects seen in *sponge* embryos are due to an actin-dependent defect rather than some alternative requirement for the *sponge* product.

While Zalokar and Erk (1976) and Edgar et al. (1987) limited their analyses to embryos in cycles 13 and 14, we have made a careful study of the *sponge* phenotype throughout the syncytial blastoderm cell cycles. Embryos from cycles 10 and 11 lack actin caps and metaphase furrows but have nuclei with normal morphologies and positions. By comparison, the nuclei of cycle 12, 13, and 14 embryos are quite aberrant. The data indicate that actin caps are not absolutely required to keep nuclei in the cortex nor are metaphase furrows absolutely required to ensure the fidelity of nuclear division. Nevertheless, it appears that these structures do have functions in the syncytial blastoderm embryo. The unusually close proximity of neighboring nuclei in cycle 11 *sponge* embryos may reflect a role for actin caps in the spacing of interphase nuclei within the apical cytoplasm. The actin metaphase furrows surely prevent the nucleation of mitotic spindles between inappropriate centrosomes. This function only appears to be critical when nuclear density is high and neighboring nuclei are closely apposed. The displacement of nuclei from the cortex of *sponge* embryos appears to be a consequence of their having undergone an abnormal division rather than their lack of a stabilizing actin cap. This is supported by the fact that all nuclei in *sponge* embryos lack actin caps but only polyploid and aneuploid nuclei are displaced from the cortex. In addition, Sullivan et al. (1990) demonstrated that in *daughterless-abo-like* embryos, which form normal actin caps, the products of an imprecise division were observed leaving the apical cytoplasm during the following nuclear cycle(s).

### *The Hexagonal Array of Actin Present in Cycle 14 Embryos Can Be Formed Independently of Actin Caps and Metaphase Furrows*

The relationship between the actin structures present in syn-

cytial blastoderm embryos and the roughly hexagonal actin network that subdivides the embryo into individual cells is unclear. During cycles 11, 12, and 13, actin filaments are rearranged in a cell cycle-dependent fashion: they form actin caps above the interphase nuclei and metaphase furrows between the dividing nuclei. During the prolonged interphase of cycle 14, actin is reorganized from caps into an array of interlinking hexagons. In both cases, the reorganization of actin involves the expansion and juxtaposition of actin caps. Based on this similarity, Simpson and Wieschaus (1990) proposed that the cycle 14 actin reorganization is controlled, at least in part, by the same processes that orchestrate the actin rearrangements during the earlier cell cycles. One might therefore expect that actin caps and metaphase furrows are prerequisites for the formation of the actin hexagonal network that mediates cellularization. However, our results demonstrate that *sponge* embryos, which never organize actin caps or metaphase furrows, can form cellularization competent hexagonal arrays of actin. Therefore, the formation of a hexagonal network must not depend on the *sponge* product or the prior organization of actin into caps or furrows. The presence of an invaginating hexagonal array of actin in *sponge* embryos confirms that the *sponge* mutation does not affect the ability of actin filaments to form ordered structures or to participate in contractile processes.

Rice and Garen (1975) reported that embryos derived from mothers homozygous for *mat(3)6*, which is allelic to *sponge*, cellularized only at their poles. Thus, they postulated that the *sponge* product was responsible for the cellularization of the equatorial regions of the blastoderm. In contrast to this earlier report, our results indicate that the *sponge* product is required during the syncytial blastoderm stage and that the cellularization abnormalities are most likely secondary consequences of these earlier defects. Although the greatest extent of cellularization occurs in the polar regions, we have observed cells formed in the equatorial portions of *sponge* embryos. Our analysis indicates that the preference for the poles reflects the fact that nuclei are least abnormal there rather than suggesting differing regional requirements for cellularization. The observed lower frequency of telophase fusions in the polar regions may be due to the curvature of the pole: not only does the nuclear density appear lower there, but adjacent nuclei rounding the pole are dividing in different planes and are thus less likely to share a mitotic spindle.

Our examination of the phenotype of *sponge* embryos has provided insight into the process of actin cap formation and the function of actin caps and metaphase furrows. However, the interactions between centrosomes and the actin-based cytoskeleton are far from understood. In addition, the mechanism for the cycling of actin structures in syncytial blastoderm embryos remains undefined. Continued investigation into the nature of the *sponge* gene product as well as the 13D2 protein and other components of the actin cap may aid our understanding of these processes.

We gratefully acknowledge the contributions of Steven Barr, Kate Harding, Eyal Schejter, and Lesilee Simpson Rose to the initial stages of this work. Christiane Nüsslein-Volhard provided us with six *sponge* alleles; Kathryn Anderson made deficiency chromosomes in the *sponge* region available to us; Doug Kellogg sent us DMAP 190 antibody for the visualization of centrosomes; and Gordon Gray supplied the fly food. We thank Robbie Hopmann, Kathy Kellerman, Val Mermall, Cordelia Rauskolb, Tito Serafini, Eyal Schejter, and Trudi Schüpbach for their valuable comments and suggestions regarding the manuscript.

Funding for this research came from National Institutes of Health grants 5R37HD15587 (to E. F. Wieschaus) and GM43607 (to K. G. Miller). K. G. Miller also received a Washington University BRSG grant. M. A. Postner was supported in part by a National Science Foundation graduate fellowship.

Received for publication 30 June 1992 and in revised form 19 August 1992.

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