RAPID COMMUNICATIONS

Myosin Rings and Spreading in Mouse Blastomeres

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ABSTRACT The relationship between myosin organization and cell spreading in the preimplantation mouse embryo was studied by indirect immunofluorescence in embryos cultured on lectin-coated substrates. Binding of cell surface polysaccharides to substrate-bound concanavalin A and wheat germ agglutinin induced changes in myosin distribution that resembled those which occur during cell-cell contact interaction. This involved an initial loss of myosin from the contact region that was associated with the development of stable cell-substrate attachments. In addition, a ring of myosin was formed along the edge of the cells' contact to the substrate. The presence of such a ring may be related to the potential for subsequent cell spreading. A myosin ring was also identified in the apical junctional region of the outer morula cells where it similarly separated the cell periphery into contacted and free peripheral domains. Following these changes in myosin organization the embryos spread on the substrate by extension of lamellipodia. These movements were coupled to the dissolution of the myosin ring and the reorganization of myosin into filament bundles. The sequence of changes in the pattern of myosin distribution suggests that contact regulation of myosin organization plays an important role in controlling the spreading behavior of blastomeres and perhaps more generally in the organization of cells into epithelia.

A major morphological change takes place in the eight-cell mouse embryo when the rounded blastomeres spread and flatten against each other. This process of compaction has an important influence on blastocyst formation and differentiation of the epithelial trophoblast. It involves active cell movement (15) and appears to be related to changes in adhesiveness (14, 15, 25) and to cytoskeletal reorganization (6, 7).

The cortex of compacting blastomeres contains a network of microfilaments (7) and immunofluorescent localization shows a continuous layer of cortical actin (16). Myosin, on the other hand, exhibits a polarized cortical distribution (22). It forms a continuous cortical layer in the uncontacted apical region of the cells and is not detectable in regions of cell contact. The loss of myosin from the contact regions is mediated by contacted interaction between the blastomeres and may play a role in the development of stable cellular associations (23). Contact modulated loss of myosin begins in the two-cell embryo and continues through the morula stages (23). It is therefore unlikely to serve as the only indication of myosin involvement during compaction. If myosin has a specific role in compaction one might expect to see a new pattern of myosin organization in which one element would be the missing patch of cortical myosin.

The present study was designed to see if spreading is associated with such a specific pattern of myosin organization. Blastomeres spread on lectin coated substrates (14) and this system was adapted to examine the temporal relationship between myosin organization and spreading. The results show that binding of cell surface polysaccharides to substrate-bound concanavalin A (Con A) and wheat germ agglutinin (WGA) induces changes in myosin organization that resemble the changes which occur during cell-cell interaction (23). This involved an initial loss of myosin from the contact region, a response which seems to be related to the stabilization of cell-substrate adhesion. Secondly, a ring of myosin developed at the edge of the embryo's contact with the substrate. A myosin ring was also identified in the apical region of the outer cells of the morula where the junctional complexes are in the process of formation. The presence of such myosin rings may be related to a developing capacity to undergo active spreading movements. Thirdly, the active movements by the substrate-bound embryos took the form of lamellar projections. Their formation was associated with the dissolution of the myosin ring and the appearance of myosin-containing filament bundles.

MATERIALS AND METHODS

Collection of Embryos: 5-7-wk-old randomly bred ICR mice (West Seneca Breeding Facility, Roswell Park Memorial Institute, Buffalo, NY) were superovulated by intraperitoneal injection of 5 I.U. each of pregnant mare's serum (PMS) (gestyl, Organon Diagnostics, West Orange, NJ) followed by human chorionic gonadotropin (HCG) (pregnyl, Organon) 48 h later and mated with CB6F1/J males (Jackson Labs, Bar Harbor, ME). Two-cell embryos were recovered at 7-9 a.m. on the second day of pregnancy and eight-cell embryos

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Culture Conditions: Zona pellucidae were removed with acid Tyrode's solution (pH 2.5) for 15-30 s followed by rinsing in a large volume of BSS. Some two-cell embryos were dissociated in calcium-free BSS. Two-cell embryos were cultured for 4-6 h and eight-cell embryos overnight on coverslips in modified egg culture medium (24) in a humidified atmosphere of air and CO2 at a medium pH of 7.3.

Preparation of Lectin and Polylysine-coated Coverslips: Con A (Sigma #C7275; Sigma Chemical Co., St. Louis, MO) and WGA (Sigma #L0636; Sigma Chemical Co.) coated coverslips were prepared by incubating acid cleaned coverslips in lectin (5 mg/ml H2O) for 2 h at 4°C and rinsing 5 times with H2O. Acid cleaned and derivatized coverslips were coated with polylysine as described by Wettsell and Filbert (27).

Immunofluorescence Microscopy: Embryos were permeabilized with 0.02% saponin, fixed in cold methanol, and stained by indirect immunofluorescence for myosin as previously described (22). Antimyosin serum was raised against human platelet myosin (8) and was a gift of K. Fujiwara (Harvard Medical School, Boston, MA). Controls for antibody specificity were the omission and preabsorption of the primary antibody as described previously (22). The embryos were examined in a Zeiss Photomicroscope II equipped with epifluorescent illumination. Coverslip cultures of the embryos have the advantage of allowing en face views of the contact region and are also convenient for making "optical sections" to compare the free and contacted regions of the same embryo. Photographs were taken with Kodak Tri-X film and developed.

RESULTS

Myosin Distribution in Adherent Two-Cell Embryos

Two-cell embryos were characterized by the presence of a cortical layer of myosin in the outer regions of the blastomeres and an absence of myosin in the region of cell-cell contact as seen in Fig. 1a where the embryo is viewed perpendicularly to the plane of contact. This distribution of myosin depends on continuous contact interaction between the cells (23). Embryos were cultured on Con A- and WGA-coated coverslips to see whether attachment to lectin-coated substrates mimics the effect of intercellular contact in the sense of inducing a similar reduction in cortical myosin from the region of cell-substrate contact. As illustrated in en face views of the contact regions in Fig. 1, b and c, the results of these experiments show that substrate binding does in fact stimulate loss of cortical myosin from the contact region. Adhesion also induced the formation of a ring of myosin, subadjacent to the cortical layer on the cytoplasmic side, surrounding the contact region (Fig. 1, b and c). These alterations in myosin organization had no discernible effect on the distribution of myosin in the free (unattached) region of the embryo (Fig. 1d).

Isolated blastomeres underwent a similar cortical reorganization on Con A- and WGA-coated coverslips, losing myosin from the contact region and forming a myosin ring that separated the contacted from the free periphery of the cell (Fig. 1e). Starting at ~6 h in culture the contact regions gave rise to small lamellar projections at the same time that adjacent parts of the myosin ring underwent dissolution and the contact region became filled with myosin-lined vesicles (Fig. 1f).

To determine if the spreading reaction and the changes in myosin distribution were due to an actual binding of cell surface polyelectrolytes to the substrate-bound lectins the embryos were cultured in the presence of Con A and WGA specific sugars. Con A has a high affinity for α-D-mannose and D-glucose and WGA for N-acetyl-D-glucosamine and its disaccharide N,N-diacytethyl chitobiose (21). Embryos on Con A-coated coverslips were therefore cultured in the presence of 50 mM α-methyl-mannoside (Fig. 1g) and embryos on WGA-coated coverslips in the presence of 50 mM N,N-diacytethyl chitobiose (not shown). These embryos showed minimal or no detectable signs of myosin redistribution and frequently detached from the coverslips during the permeabilization treatment.

Embryos attached to polylysine-coated coverslips also failed to exhibit any significant changes in myosin distribution (Fig. 1h). The contact region remained relatively small, continued to show a diffuse distribution of myosin, and the embryos usually detached from the coverslips during permeabilization. Embryos cultured on untreated coverslips behaved in a similar fashion.

Myosin Distribution in Adherent Morulae

Morulae were characterized by the presence of myosin in the outer blastomeres. These cells exhibited a cortical myosin layer in the apical region of the cells and an additional concentration of myosin in the region of the apical junctions (Fig. 2a). Embryos do not normally show myosin in regions of cell contact and the presence of residual amounts of myosin in contacted regions reflects an incomplete recovery from the acid Tyrode treatment used to remove the zona pellucida (23). Focusing on the free (unattached) periphery of the embryo shows that myosin has a diffuse distribution in the cortical layer and is concentrated in continuous rings in the apical region of the outer blastomeres (Fig. 2b). Morulae cultured on polylysine-coated coverslips usually detached from the coverslip during permeabilization. The few that remained attached showed a limited degree of flattening on the substrate with no apparent changes in myosin distribution in the contact region (Fig. 2c). In contrast, embryos attached to WGA- (Fig. 2d) and Con A- (Fig. 2e) coated coverslips underwent an extensive flattening reaction that was correlated.

### Figure 1: Localization of myosin in two-cell embryos and blastomeres attached to lectin and polylysine-coated coverslips. The diagram illustrates the planes of focus. Contact region, plane X'; embryo midsection, plane X'"; free (unattached) surface, plane X"'. The hatched areas indicate staining for cortical myosin; the clear areas an absence or reduced staining. The broken line marks the locus of the myosin ring. (a) Embryo midsection. Myosin is concentrated in the outer cortices and is not detectable in the region of cell contact. (b-c) The contact region of embryos cultured on WGA- (b) and Con A- (c) coated coverslips. Myosin is lost from the contact areas and is concentrated in a ring around the perimeter of the contact region. (d) The free surface of an embryo cultured on a Con A-coated coverslip. Cortical myosin has a diffuse homogenous distribution. (The embryo is viewed with one blastomere in front of the other and the bright fluorescent margin of the anterior blastomere should not be confused with the contact region.) (e-g) The contact region of isolated blastomeres cultured on Con A-coated coverslips. (e) Myosin is lost from the contact region and forms an asymmetric ring around the perimeter of the contact region. (f) A lamellar projection is growing out from the contact region and the adjacent part of the myosin ring has disappeared (arrow), (g) Blastomere cultured in the presence of 50 mM methylmannoside shows minimal redistribution of cortical myosin. (h) The contact region of a blastomere cultured on a polylysine-coated coverslip. There is no discernible redistribution of myosin. × 500.
with loss of myosin from both the cortical layer and the apical rings in the contact region. Maximal flattening of the intact embryo was associated with the development of a multicellular ring of myosin around the contact region with segments of the ring derived from adjacent blastomeres (Fig. 2f). The ring was breached (Fig. 2f) and subsequently disappeared (Fig. 2g) as individual cells and then the entire cell population formed lamellar projections with typical myosin containing filament bundles. The specificity of these reactions was tested by culturing embryos on Con A- and WGA-coated substrates in the presence of 50 mM α-methyl-mannoside (Fig. 2h) and N,N′-diacetylchitobiase (not shown), respectively. The embryos usually detached from the substrate during permeabilization and did not show any apparent redistribution of myosin from either the cortical or the apical junctional regions.

**DISCUSSION**

*From Adhesion to Spreading*

The present study shows that attachment of embryos to lectin-coated substrates induces changes in myosin organization that mimic those that occur when cells develop contact relations with each other (23). Both types of contact stimulated a loss of cytoskeleton-bound myosin from the contact region. This reaction appears to be specifically evoked by the binding of cell surface polysaccharides to the Con-A- and WGA-coated substrates since the response was prevented by the addition of specific sugar inhibitors. These observations lend support to the immunological evidence (4, 13) which suggests that glycoprotein receptors are involved in adhesive interactions between blastomeres. Attachment of polylysine-coated substrates failed to induce any significant changes in myosin distribution. These embryos were also much more susceptible to detachment from the coverslips after detergent treatment than were embryos attached to lectin-coated substrates. A similar correlation between the fragility of cell contacts and the presence of myosin on the one hand and the development detergent-resistant cell contacts and loss of detectable myosin on the other hand was characteristic of cell-cell contacts (23).

Embryos attached to lectin-coated substrates formed a ring of myosin around the contact region. Each blastomere of the two-cell embryo exhibited such a ring around its missing patch of cortical myosin while the morula formed a single multicellular ring that was actually composed of adjacent segments from the neighboring cells. This ring encircled a contact region from which both the cortical layer of myosin and apical myosin rings had been lost.

![Figure 3 Diagrammatic representations of myosin distribution in the compacted morula. Hatched areas indicate staining for cortical myosin; clear areas an absent or weak staining for myosin. The broken lines mark the presence of a myosin ring. (a) Surface view of the free (unattached) periphery: there is a cortical layer of myosin and underlying myosin rings which are located in the apical junctional regions. (b) The outer layer of cells viewed from inside the embryo: an apical myosin ring divides each cell into two domains with the apical (uncontacted) part of the cell exhibiting a cortical layer of myosin and the contacted lateral and basal cell peripheries depleted of cortical myosin.](image)

Following these changes in myosin distribution the blastomeres spread on the substrate by means of lamellipodia (see also reference 14). The movement of blastomeres on lectin-coated substrates thus resembles the mode of spreading of blastomeres against each other which involves similar cytoplasmic processes (3, 15, 26). The development of lamellipodia was associated with further changes in myosin organization that resulted in the dissolution of the myosin ring and the formation of myosin-containing filament bundles.

In summary, the adhesion of embryos to lectin-coated substrates induces partition of the embryo periphery into two domains, separated by a ring of myosin, in which the free (uncontacted) region is characterized by a cortical layer of myosin and the contacted region by an absence of detectable myosin. The peripheral organization of the adherent embryo appears to be analogous to that of the outer cells of the morula. In these cells an apical myosin ring separates the free apical region with its layer of cortical myosin from the lateral and basal cell borders which are in contact with neighboring cells and lack myosin (compare Figs. 1, 2, and 3).

**The Myosin Ring**

The myosin ring of the outer cells of the morula is located at the level of the apical junctional complex that encircles each cell and attaches it to the neighboring cells (5, 17). In this respect the outer layer of the morula exhibits a typical epithelial morphology (5) and it seems likely that the apical myosin ring of the blastomeres corresponds to the apical...
contractile ring of filaments which is associated with the junctional complex of epithelial cells. Such a contractile ring has been described in sheets of pigmented epithelium (19), and in the intestinal brush border (1, 12) where myosin has been specifically localized in the adherens zone (2, 11). The intimate relationship between the myosin ring and the zonula adherens might have its counterpart in the substrate-bound embryo. This would be the case if, for example, the myosin ring developed in conjunction with a ring of adhesion sites. Such a relationship would be consistent with the structural and compositional similarity of the zonula adherens and the adhesion plaques whereby fibroblasts attach to the substrate (9, 10).

The function of the epithelial myosin ring is controversial but it appears to influence the movement of microvilli (2, 20) and may play a structural role in maintaining tension between adjacent cells (11). The possibility that myosin rings may regulate microvillar movement in the embryo is particularly intriguing in view of the evidence that a ring of lateral microvilli mediates cell surface recognition and attachment between adjacent blastomeres during compaction (3, 26). On the other hand, a structural function in the embryo may indicate a role for myosin rings in maintaining the integrity of the outer layer of blastomeres and the rounded shape of the morula. Loss of the apical myosin rings during attachment of the embryos to the substrate could then serve as a mechanism to relieve contractile forces that might otherwise prevent the embryo from flattening.

The observations of the present study suggest another possible role for the myosin ring, namely, as a structure whose presence may be related to the potential for spreading. This is inferred from the following sets of observations: First, in the two-cell embryo a myosin ring was formed by each blastomere at the site of its attachment to the lectin-coated substrate whereas no such enhanced concentration of myosin was observed around the contact region between the blastomeres. Secondly, in the morula stages, myosin rings were formed by the outer blastomeres and not by the inner blastomeres. It appears, therefore, that a myosin ring is only produced by cells that have a polarized distribution of myosin and where, in addition, the myosin-free domain has the capacity for active spreading. This would account for the fact that myosin rings do not normally develop in the two-cell embryo where the blastomeres are polarized but lack the ability to spread against each other, and why myosin rings first appear in the polarized outer cells of the morula when the cells are acquiring the capacity for spreading. In this view the formation of a multicellular ring by the adherent morula is related to the potential of the contacted cells to undergo active spreading movements on lectin-coated substrate.

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