Localization of cytoskeletal proteins in preimplantation mouse embryos

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SUMMARY

The immunofluorescence technique was used to detect the presence and distribution of actin, alpha-actinin, tubulin and 10 nm filament protein in early mouse embryos. Actin and alpha-actinin stainings showed a distinct concentration to a peripheral layer in the cleavage-stage blastomeres and in trophectoderm cells. Dots of fluorescence appeared in this cortical staining pattern. The distribution of tubulin staining in the blastomere cytoplasm was relatively even with apparent concentration at the perinuclear region and frequently at wide intercellular contact areas. 10 nm filament protein was distributed evenly in the blastomere cytoplasm without cortical concentration of the label. At the blastocyst stage, the trophectoderm cells in blastocyst outgrowths in vitro developed well organized cytoskeletons including both microfilament, microtubule and 10 nm filament elements. Comparable structures were not observed in blastocysts in vivo, or in late hatched blastocysts cultured in suspension. The morphogenetic significance of the observations is discussed.

INTRODUCTION

Microtubules, microfilaments and intermediate filaments are the major fibrous elements recognized by electron microscopy in the cytoplasm of eukaryotic cells (Goldman & Knipe, 1973). These structures are thought to be involved in cell movement, cell anchorage to the substratum, the maintenance of cell shape, and the movement of membrane proteins. The cytoskeletal components constitute organized intracellular patterns which can be demonstrated relatively easily in flat cells grown in culture with immunofluorescence techniques (Lazarides & Weber, 1974; Weber, Pollack & Bibring, 1975; Osborn, Franke & Weber, 1977).

It is well established that morphogenetic cell movements have a central role in embryogenesis. It has been suggested that both microfilaments and microtubules are involved in the morphogenetic processes of neurulation, gastrulation, invagination of the lens placode and branching of the epithelial rudiment in glandular morphogenesis (for review, see Spooner, 1974; Trinkaus, 1976). How-

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ever, cell locomotion mechanisms in multicellular organisms are poorly understood, and the above associations have generally been based on the ultrastructural demonstration of microfilaments and microtubules in sectioned material and on studies with cytochalasin B and colchicine. At present, few immunofluorescence observations of cytoskeletal structures have been presented for intact multicellular organisms, and it has been difficult to build up a picture of the cytoskeleton pattern in the whole cell from electron microscopy sections.

During the early cleavage and the blastocyst stages of mouse embryogenesis, several important morphogenetic movements take place. In the 8-cell mouse embryo, the contact areas between the individual blastomeres dramatically increase leading to 'compaction' of the embryo. This phenomenon, considered to be essential for the segregation of inner and outer cells of the embryo (Tarkowski & Wroblewska, 1967) is prevented by the microfilament-disrupting agent cytochalasin B (Ducibella & Anderson, 1975). Furthermore, cytochalasin B has been reported to inhibit the morula to blastocyst transformation in the mouse embryo (Granholm & Brenner, 1976). The ultrastructural localization of microfilaments and microtubules (Ducibella, Ukena, Karnovsky & Anderson, 1977) and the synthesis of actin and tubulin (Abreu & Brinster, 1978a, b; Schultz, Letourneau & Wassarman, 1979) in early mouse embryos have been well documented. In the present study we have used immunofluorescence techniques to monitor the cytoskeletal components during cleavage and the blastocyst stages of the mouse development in vivo. In addition, we have studied the cytoskeletal structures in trophectoderm cells during trophoblast outgrowth in vitro.

MATERIALS AND METHODS

Embryos. The embryos used in this study were from natural matings and were A2G, 129J/SV, C3H, (129J×C3H) F1, (C57Bl/6×CBA/Ha) F2 and CFLP outbred stock (Anglia Laboratory Animals Ltd.). No obvious differences between strains were noticed and the results are therefore described together.

Embryo culture. The embryos were dissected into prewarmed pre-equilibrated Whitten's medium (1971). The zona pellucida was removed either by dissolving it with acid Tyrode's solution at pH 2.5 (Nicolson, Yanagimachi & Yanagimachi, 1975) or by digesting it partially with pronase (Calbiochem Co., U.K.) and thereafter pipetting it off the embryo (Mintz, 1967). Before fixation, the zona-free embryos were cultured for 1–2 h in Whitten's medium in a humidified gas mixture of 5% CO2, 5% O2 and 90% N2 at 37 °C.

For in vitro blastocyst outgrowths, the blastocysts were collected late on the fourth day (the day of appearance of the vaginal plug being the first day of gestation). The embryos were cultured in alpha-medium (Stanners, Eliceiri & Green, 1971; lacking nucleosides and deoxynucleosides) with 10% heat-inactivated foetal calf serum (Flow Laboratories, Irvine, Scotland) in a humidified gas mixture of 5% CO2 in air at 37 °C. For culture, the embryos were placed
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in the middle of 22 × 22 mm coverslips lying in 30 mm diameter Petri dishes. The coverslips were previously treated with 0.1 % (w/v) gelatin (Type I, swine skin collagen, Sigma Chemical Co.) at 4 °C for 60 min. Most of the embryos hatched from their zona pellucida and attached to the coverslips on the second day in culture. The resulting blastocyst outgrowths were fixed after 1–5 days from the beginning of cultivation; the medium was changed on day 4. Blastocysts from the same matings were cultured in suspension in bacteriological dishes (Sterilin Ltd, Richmond, Surrey, U.K.). These blastocysts were fixed after 1–3 days in culture.

Fixation. The fixation and immunofluorescence-staining steps were carried out in two ways: (1) The embryos were pipetted throughout the procedure with a siliconized (Repelcote, BDH, U.K.) mouth pipette. Fixation with formaldehyde or glutaraldehyde and rinsings with PBS were done in dishes coated with 1 % (w/v) agar in 0.6 % (w/v) NaCl; methanol and acetone treatments were performed in glass dishes; incubations with the antibodies were conducted in bacteriological dishes in microdrops under paraffin oil (Boots Pure Drug Co., U.K.). These procedures reduced the number of embryos which stuck to the pipette or dish and were lost. (2) After or immediately before fixation the embryos were pipetted onto a coverslip treated with 0.1 % (w/v) poly-L-lysine in water (McKeehan & Ham, 1976). The embryos stuck on this surface and were carried through the following steps on the coverslips.

Before fixation, the embryos were rinsed in prewarmed PBS (solution A of Dulbecco & Vogt, 1954) or in Whitten’s medium without albumin. The preparation for immunofluorescence microscopy was performed by one of the following procedures. All steps were at room temperature unless otherwise stated. (1) 2 % (w/v) paraformaldehyde or 3.7 % (w/v) formaldehyde in PBS or in 0.1 M-Na-cacodylate buffer, pH 7.2 for 3–30 min; rinse in PBS; methanol at −20 °C for 5–15 min; acetone at −20 °C for 0.5–3 min; PBS. The acetone step was sometimes omitted. The embryos were sometimes air-dried after the acetone step. (2) As procedure 1, but PBS was replaced with the buffer solution of Small & Celis (1978a). (3) As procedure 1, except that the formaldehyde step was omitted. (4) 1.5 % (w/v) glutaraldehyde in PBS for 7–20 min; methanol at −20 °C for 15 min; rinse in PBS; 0.5–1 mg/ml NaBH₄ in PBS for 20 min; PBS (Weber, Rathke & Osborn, 1978). (5) Brief formaldehyde fixation as in procedure 1; 0.1–1.0 % (w/v) Triton X-100 or NP 40 in PBS, at 37 °C for 0.5–20 min; methanol at −20 °C (sometimes omitted); rinse in PBS. (6) As procedure 5, but the detergent step of 0.5–10 min preceded the fixation with formaldehyde (sometimes omitted) and methanol. (7) As procedure 6, but the detergent step was in the ‘stabilization buffer’ (Osborn & Weber, 1977) and was preceded by 1–2 min in the stabilization buffer at +37 °C.

For stainings with anti-actin and anti-alpha-actinin, variations of all the above procedures gave basically identical results, and the procedures 1, 2 and
were used most frequently. For stainings with anti-tubulin, the procedures 2, 4 and 7 appeared to give the best results as judged from the blastocyst outgrowth stainings. For 10 nm filaments, the procedures 2 and 3 were used most frequently, but the procedures 1, 5 and 6 were also employed.

Scanning electron microscopy (SEM, methods of Graham & Lehtonen, 1979) was used to assess the cell morphology after the fixation procedures used for immunofluorescence microscopy.

**Immunofluorescence microscopy.** The staining procedures for indirect immunofluorescence were as follows. The first antibody was applied for 30–60 min at +37 °C in a humidified chamber. After thorough rinsing in PBS, fluorescein-labelled goat anti-rabbit IgG (Nordic Immunological Laboratories, Tilburg, The Netherlands) (diluted 1/30–1/50 with PBS or the buffer solution of Small & Celis, 1978a), was applied for 30–60 min at +37 °C in a humidified chamber. After thorough rinsing in PBS the samples were mounted in 80 % glycerol in PBS on microscope slides. The slides were viewed with epifluorescence optics in a Zeiss photomicroscope. Photographs were recorded on Ilford HP5 film.

**Antibodies.** The methods for raising the antibodies against actin, alpha-actinin, tubulin, and smooth muscle 10 nm filament protein and their purification and characterization are described elsewhere (Badley et al. 1978). The antisera were used at protein concentrations ranging from 1·4 mg/ml to 2·8 mg/ml. Further purification of the antibodies was achieved by affinity purification on antigen-Sepharose 4B columns prepared and used essentially according to Jockusch, Kelley, Meyer & Burger (1978). The affinity-purified antibodies were

**Figures 1–8**

Figs. 1–2. A 2-cell-stage embryo stained with antibody to actin, focused at the middle (Fig. 1) and close to the lower surface (Fig. 2) of the embryo. Both the cells and the polar bodies have a peripheral actin layer, which contains apparently submembranous dot-like structures.

Fig. 3. An early 8-cell-stage embryo, anti-actin staining. Cortical concentration of the label is relatively strong and includes dot structures. PB, polar body.

Fig. 4. A compacted 8-cell-stage embryo, anti-actin staining. The pattern of fluorescence is comparable to that in Fig. 3. PB, polar body.

Fig. 5. The upper surface of a blastocyst fixed after 27 h in culture, anti-actin staining. The borders of the trophectoderm cells show a relatively strong affinity for the label. The ICM (not in the focal plane) is situated in the lower part of the picture and increases the actin-specific background fluorescence.

Fig. 6. A higher magnification from the lower surface of the blastocyst in Fig. 4. A possible microfilament bundle is indicated (arrow).

Fig. 7. A blastocyst outgrowth after 2 days in culture, anti-actin staining. The trophectoderm cells show microfilament bundles orientated in the direction of cell migration.

Fig. 8. A blastocyst outgrowth after 5 days in culture, anti-actin staining. Microfilament bundles similar to those in Fig. 6 are present. In addition, the edges of the large trophectoderm cells contain microfilaments orientated parallel to the cell border.
used at protein concentrations ranging from 60 µg/ml to 120 µg/ml. The controls included (a) the omission of the first antibody, (b) the replacement of the first antibody with a non-immune serum or (c) with affinity-absorbed sera at concentrations equal to the concentration of the corresponding antibody. The controls always lacked the specific green fluorescence. They usually showed a weak yellowish fluorescence which disappeared within a few seconds and which was too weak to photograph. For examining the behaviour of 10 nm filaments some blastocyst outgrowths were cultured in 10 µg/ml colchicine in growth medium for 17 h immediately before fixation (Starger & Goldman, 1977).

RESULTS

Actin

Antibodies against smooth muscle actin showed affinity for mouse embryos starting from the 1-cell stage. The staining pattern was essentially the same in all the cleavage-stage embryos. Usually, a bright peripheral ring of fluorescence was seen in addition to a relatively weak cytoplasmic actin-specific fluorescence (Figs. 1–4). The cortical concentration of actin-containing material appeared to be located immediately beneath the cell membrane and it occasionally

Figures 9–16

Fig. 9. A 2-cell-stage embryo stained with antibody to alpha-actinin. As compared to the anti-actin-stained embryos, the peripheral concentration of the label and the dot structures appear less conspicuous relative to the alpha-actinin-specific background fluorescence.

Fig. 10. A blastocyst after 27 h in culture, anti-alpha-actinin staining. The borders of the trophectoderm cells show a relatively strong affinity to the label. The accentuated alpha-actinin-specific background fluorescence in the upper part of the Figure represents the ICM (not in the focal plane).

Fig. 11. An early 2-cell-stage embryo stained with antibody to tubulin. Note the apparent concentration of the label to the perinuclear region.

Fig. 12. Two late 2-cell-stage embryos allowed to adhere to each other in culture, anti-tubulin staining. In addition to the cytoplasmic fluorescence, both cell contact areas show increased affinity to the label. A possible microtubular fibre structure is indicated (arrow).

Fig. 13. An 8-cell-stage embryo. The anti-tubulin specific fluorescence is relatively even with some concentration of the label to intercellular contact areas. Note the mitotic spindle in the cell beneath the polar body (PB). Inset: higher magnification of the spindle in Fig. 13.

Fig. 14. A blastocyst after 27 h in culture, anti-tubulin staining. Note the apparent perinuclear fluorescence in the trophectoderm cells. The accentuated tubulin specific background fluorescence in the upper part of the Figure represents the ICM (not in the focal plane).

Figs. 15–16. Blastocyst outgrowths, anti-tubulin staining. After 2 days in culture (Fig. 15) the first out-migrating cells show distinct microtubular figures. After 5 days in culture (Fig. 16) the microtubule bundles are well developed and often appear both above and below the nucleus. The microtubules frequently seem to originate from the perinuclear region. N, nucleus.
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seemed to be accentuated at the intercellular contact areas. When observed close to the level of the cell membrane, dots of fluorescence occurred in the peripheral staining pattern (Figs. 1–4). The density of these dots varied to some extent. The polar body was regularly fluorescent with an intensity comparable to that of the cellular staining. No changes in the staining pattern were observed upon compaction (Figs. 3–4).

At all blastocyst stages examined, the cells of the inner cell mass (ICM) showed diffuse actin-specific fluorescence. The trophectoderm cells showed apparent concentration of actin-specific fluorescence at the cell borders (Fig. 5). Occasionally, indications of possible cytoplasmic actin filaments were observed in favourable preparations, more frequently in late expanded blastocysts which were prevented from attaching to the substratum (see Materials and Methods) (Fig. 6). Usually only a few fluorescent strands without distinct or orientated patterns were seen.

In the blastocyst outgrowths, the trophectoderm cells growing flat on a glass substratum showed distinct cytoskeletal patterns. Relatively strong actin fibres oriented in the direction of cell migration were seen already in the first cells growing away from the main mass of cells on day 2 from the beginning of culture (Fig. 7). During the following days the trophectoderm cells spread more extensively on the substratum. In these cells the microfilament organization pattern usually appeared in several layers and consisted of cables orientated in the direction of cell migration and of filaments running parallel to the free edge of the cell (Fig. 8). The ICM cells in these preparations were growing in a pile and were not attached to the glass. They showed a strong actin-specific fluorescence without any observable pattern of organization.

**Alpha-actinin**

Antibodies against alpha-actinin gave a somewhat similar but more even staining pattern than those against actin. The boundaries of the cleavage-stage cells were easily visualized, but the alpha-actinin-specific background fluorescence was relatively strong. Also, the peripheral dot-like structures were less conspicuous than in the embryos stained with anti-actin (Fig. 9). The polar body was regularly fluorescent.

At all blastocyst stages examined, both the ICM and the trophectoderm cells exhibited alpha-actinin-specific fluorescence with concentration of the label in the areas of intercellular contacts (Fig. 10). Occasionally, indications of delicate, alpha-actinin-containing filaments were seen in the trophectoderm cells although photographic documentation of these filaments was difficult.

In blastocyst outgrowths, trophectoderm cells which had spread over the coverslip showed alpha-actinin-containing filamentous structures. The organization pattern of these filaments was similar to that observed in the case of actin. The ICM cells in these preparations were relatively strongly fluorescent.
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**Tubulin**

Antibodies against tubulin usually gave rise to a relatively even staining pattern within the blastomeres and polar body of cleavage-stage embryos. Sequential focusing through specimens treated with detergent before fixation (Materials and Methods, procedure 7) often gave an impression of reticular intracellular staining, although photographic documentation of this was difficult. No clear cortical concentration of fluorescence or dot-like structures comparable to those in the case of microfilament-associated proteins could be observed; rather the activity seemed to diminish towards the cell periphery so that the cell borders were often difficult to visualize (Figs. 11 and 13). Occasionally, however, the intercellular contact areas showed affinity to the antibody (Figs. 12–13). This staining pattern was frequent in the case of the widest intercellular contacts at each cleavage stage but was not observed in the contacts between newly divided cells. Apparent perinuclear concentration of the label was frequently seen (Fig. 11). As expected, the mitotic spindles showed strong affinity to the antibody (Fig. 13). Occasionally, possible individual fluorescent fibres were observed against the general background of cytoplasmic tubulin (Fig. 12).

At all blastocyst stages examined, both the ICM and the trophectoderm cells showed tubulin-specific fluorescence (Fig. 14). In the ICM cells, the cytoplasmic tubulin appeared to be relatively evenly distributed. In contrast to the case of microfilament-associated proteins, there was no concentration of fluorescence at the boundaries of the trophectoderm cells. These cells occasionally showed indications of individual weak filamentous structures which appeared to originate from the perinuclear area. Perinuclear tubulin-specific fluorescence was regularly observed (Fig. 14).

In the blastocyst outgrowths, already the first trophectoderm cells growing away from the main mass of cells showed distinct microtubular figures (Fig. 15). During the following days in culture the cells developed microtubular patterns which usually appeared in several layers (Fig. 16). The microtubules frequently appeared to originate from the perinuclear area (Figs. 15–16). The ICM cells in these preparations were strongly fluorescent without any observable intracellular organization pattern.

**10 nm filament protein**

In the cleavage-stage embryos, antibodies against smooth muscle 10 nm filament protein usually gave rise to even cytoplasmic fluorescence without noticeable cortical concentration of the label. Relatively strong perinuclear fluorescence was regularly observed (Fig. 17).

At all blastocyst stages examined, both the ICM and the trophectoderm cells exhibited 10 nm-filament-protein-specific fluorescence. The cell boundaries did
Fig. 17. A 2-cell-stage embryo stained with antibody to 10 nm filament protein. The cytoplasmic fluorescence is relatively even without cortical concentration of the label.

Fig. 18. A blastocyst outgrowth after 5 days in culture, stained with antibody to 10 nm filament protein.

not show increased affinity to the label. Perinuclear fluorescence was regularly observed in the trophectoderm cells.

In the 5-day blastocyst outgrowths, both the ICM and the trophectoderm cells exhibited 10 nm-filament-protein-specific fluorescence. Increased fluorescence in the cell boundaries was not observed. Most of the flattened trophectoderm cells contained a network of branching filaments (Fig. 18). Distinct perinuclear fluorescence was regularly seen in the trophectoderm cells (Fig. 18). In the presence of colchicine, aggregates of filaments comparable to those seen in fibroblasts (Starger & Goldman, 1977; Franke, Schmid, Osborn & Weber, 1978) were observed in some of the outgrowth cells. Filament aggregation was not correlated with the position or nuclear size of the migrating cell.

**DISCUSSION**

The decisive problem in the mechanism of blastocyst development is to understand how the individual cells move into and retain their relative positions in the cleavage-stage embryo (Wilson, Bolton & Cuttler, 1972; Graham & Deussen, 1978; Kelly, Mulnard & Graham, 1978; Graham & Lehtonen, 1979). In this paper we have studied the organization pattern of intracellular cytoskeletal proteins in early embryos in order to investigate their possible involvement in the early morphogenetic processes.

*Cytoskeleton in cleavage-stage embryos*

In this investigation, we found clear differences in the distribution of microfilament-associated and microtubule-associated fluorescence in the early
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In the cells of cleavage-stage embryos, actin was displayed as an intense cortical layer with a relative weak cytoplasmic background. Anti-alpha-actinin similarly had affinity to the cell periphery but the relative cytoplasmic fluorescence appeared stronger than in the case of actin. Anti-tubulin on the other hand, with the exception of the wide intercellular contact areas, did not show cortical affinity in the cleavage-stage cells. The cytoplasmic distribution of 10 nm-filament-protein-specific fluorescence was relatively even with some perinuclear concentration. Comparable 10 nm filament staining around the nucleus has been observed in cells in culture (Small & Celis, 1978b).

It has been shown that actin and tubulin are actively synthesized in the cleavage-stage mouse embryos (Abreu & Brinster, 1978a; Schultz et al. 1979), but no earlier immunofluorescence studies on cytoskeletal components in early mammalian embryos are available. However, sea-urchin zygotes apparently display a cortical concentration of actin (Wang & Taylor, 1978) comparable to our findings, and it has been suggested that a thin band of actin-specific fluorescence is associated with oolemma of cryosectioned rat oocytes (Amsterdam, Lindner & Gröschel-Stewart, 1977). Furthermore, mouse oocyte cytoplasm as well as the polar body and meiotic spindle apparently have affinity to anti-tubulin (Wassarman & Fujiwara, 1978).

Recent electron microscopical observations have suggested that both 2-cell- (Opas & Soltynska, 1978) and 8-cell- (Ducibella et al. 1977) stage mouse embryos have a cortical microfilament layer, whereas the microtubules are arranged more orthogonally to the cell surface. In the compacting 8-cell-stage embryos, however, the microtubules appeared to be arrayed parallel to the membrane at the cell contact areas (Ducibella & Anderson, 1975; Ducibella et al. 1977). Our observations are consistent with these suggestions: in the cleavage-stage embryos the principal microfilament-associated fluorescence appeared as a continuous cortical layer whereas peripheral concentrations of tubulin-specific fluorescence were found only at wide intercellular contact areas. Generally, we could not demonstrate clear microtubular fibre structures in the cleavage-stage embryos. This is probably due to the rounded morphology of the blastomeres which hinders the immunofluorescence visualization of individual microtubules (Osborn & Weber, 1977).

In the present study, the actin-specific and alpha-actinin-specific fluorescence usually included distinct peripheral dot-like structures. In the preparation of blastomeres for reaction with antibodies the formaldehyde-fixed cells are exposed to methanol and acetone which permeates the cell membrane (Fig. 19). Consequently, the antibody can bind to intracytoplasmic actin and to the microfilaments observed in surface microvilli (Ducibella et al. 1977). Therefore, the dots of actin-specific fluorescence may mark the position of preserved microvillar material, as reported in the case of isolated amphibian oocyte cortex (Franke et al. 1976), or they may simply reflect discontinuities in the cell membrane (Fig. 19), under which lies a layer of fluorescent material.
Fig. 19. A scanning electron micrograph of an isolated 8-cell-stage blastomere. After dissociation of the embryo, the cell was cultured for 60 min, formaldehyde fixed, treated with $-20^\circ C$ methanol, treated with $-20^\circ C$ acetone and processed for SEM. The ultrastructural preservation of the cell is poor, there are frequent discontinuities in the plasma membrane. Note the remnants of microvillar membrane protrusions.

However, the fluorescent dots (Figs. 1–4) appear to be too large to be due to microvilli (Fig. 19), and furthermore, they seem to be submembranous (compare Figs. 1 and 2). A more likely explanation is that these dots are cortical microfilament aggregates. This is consistent with the electron microscopical observation that major thickness variations occur in the peripheral microfilament layer of mouse embryos (Opas & Soltynska, 1978). Comparably, cortical microfilament aggregates have been reported in amphibian oocytes (Franke et al. 1976) and in wounded amphibian eggs (Bluemink, 1972).

Cytoskeleton in attached trophectoderm cells

In the blastocyst outgrowths, after 5 days in culture, all three cytoskeletal elements were well developed in the spread-out trophectoderm cells, and we observed distinct microfilament and microtubular patterns already in the first outmigrating cells on the first day after blastocyst attachment. This is somewhat earlier than in a previous report by Sobel, Cooke & Pedersen (1978): they used fluoresceinated myosin subfragment 1 to stain glycerinated mouse blastocyst cultures and with this method observed the first signs of microfilament organization between day 2 and 3 after attachment. Unlike the
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trophectoderm cells in blastocyst outgrowths, we did not observe well developed
cytoskeletal structures in the trophectoderm cells of late expanded blastocysts
cultured in suspension. This is consistent with the suggestion, based on studies
with normal and transformed fibroblasts, that the presence of microfilament
bundles is related to the shape of the cell and its flattening on the substratum
(Willingham et al. 1977). In a rather similar way, microfilament bundles appear
when small rounded embryonal carcinoma cells form well spread differentiated

Recent work has shown the presence of different forms of 10 nm filaments
in different cell types (Franke et al. 1978). The staining of most of the trophecto-
dermal, 5-day outgrowth cells with our anti-chicken gizzard 10 nm filament
antibodies suggests that these cells contain filaments analogous to those we
have found in chick embryo fibroblasts, BHK cells and a subpopulation of rat
embryo fibroblasts (R. A. Badley, unpublished). Furthermore, some of the
blastocyst outgrowth cells showed colchicine-induced filament aggregates, which
suggests that these filaments are not of the pre-keratin type, reported to be
predominant in many epithelial cells (Franke et al. 1978), but rather of the
type seen in fibroblasts (Starger & Goldman, 1977).

Possible biological significance of the observations
The developmental significance of cytoskeletal structures in early embryos
is unknown. After using several different preparation methods we think that
mouse cleavage-stage embryos do not contain orientated bundles of cytoskeletal
structures. This is perhaps not surprising as these cells are spherical. Important
morphogenetic movements occur during the cleavage period, and these move-
ments appear to be connected to cell divisions (Graham & Deussen, 1978;
Graham & Lehtonen, 1979). These cell movements may depend on the peri-
pheral concentration of microfilaments observed in the cleavage-stage blasto-
meres. A dense microfilament layer possibly provides the cell cortex with
mechanical rigidity. During cytokinesis cell contacts appear to restrict the
direction of cell elongation (Graham & Lehtonen, 1979) and the action of
these contacts may well be mediated by the stiffness of cytoskeletal elements.
By analogy with adult cells, it is likely that changes in blastomere agglutin-
ability (Rowinski, Solter & Koprowski, 1976) and cell cycle changes in blasto-
mere contact (Lehtonen, in preparation) are all mediated by elements of the
cytoskeleton.

At the blastocyst stage the trophectoderm cells are capable of developing
a well organized cytoskeleton. However, the formation of orientated bundles
of cytoskeletal elements appears to be triggered by adhesion to a substratum,
as corresponding structures were not observed in blastocysts cultured simulta-
neously in suspension. In vivo, comparable dynamic changes in the cyto-
skeletal organization may be important in the interactions of trophectoderm
cells with the uterine epithelium during implantation.

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REFERENCES


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