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Development of Cellular Polarity of Hamster Embryos during Compaction[1]

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ABSTRACT

Development of cellular polarity is an important event during early mammalian embryo development and differentiation. Blastomeres of hamster embryos at various stages were examined by scanning electron microscopy (SEM) and immunocytochemical staining. SEM observations revealed that 1- to 7-cell-stage embryos showed a uniform distribution of microvilli throughout the cell surface. Microvillous polarization was initially noted in the blastomeres (10–35%) of 8-cell-stage embryos. The polarized microvilli were observed mostly in the basal region of cell-cell contact and occasionally at the apical, outward-facing surface of the blastomere. Fluorescein-isothiocyanate-conjugated concanavalin A failed to reveal any polarity in the blastomeres regardless of the stages of the embryos. Actin staining showed that microfilaments were present beneath the cell surface, and in addition, areas of cell contact were more heavily stained, indicating a thick microfilament domain. Microtubules were located throughout the cytoplasm and were heavily concentrated near the nucleus during interphase, although they became redistributed in the region of the mitotic spindle during karyokinesis. The position of nucleus changed from the cell center to the apical, outward-facing surface of the cell, and it distanced itself from the basal microvillous pole. It is suggested that the changes in the cell surface and nuclear position are the first manifestations of cell polarity in peri-compacted hamster embryos, which appear as early as the 8-cell stage; furthermore, the outward migration of the nuclei may parallel the redistribution of microtubules in the cytoplasm.

INTRODUCTION

During early development of mammalian embryos, considerable cell surface and cytoplasmic changes occur around the time of embryo compaction (peri-compaction). These phenomena have been referred to as cellular polarization. Polarization was characterized by microvilli distribution on the individual blastomeres of the peri-compacted embryos [1–4], by the restriction of surface fluorescent-ligand or antiserum binding sites [2–8], and by localization of some enzymes in the cytoplasm [9, 10]. Reeve and Kelly [11] observed that nuclei were randomly located in the blastomeres of the early 8-cell mouse embryo but migrated to a basal position in each cell as the 8-cell-stage progressed. Also, in the mouse, mitochondria and microtubules become aligned in the cytocortex parallel to the plasma membrane in the cell-cell contact region between blastomeres [1]. However, microtubules in those blastomeres were found randomly arranged near the apical microvilli pole of the cell [1]. In mice, cytoplasmic actin, which is homogeneously distributed in the early 8-cell embryo, became excluded from contact regions and was restricted to the apical portion of each blastomere during the 8-cell stage [12]. During subsequent cell divisions, the blastomeres develop to inside nonpolar and outside polar cells in the mouse blastocyst, giving rise to two differentiated cell lineages: the inner cell mass and the trophectoderm [13–21].

While embryo polarity has been studied extensively in mice, information on cell polarization of embryos in other animals is very limited. Microvillous polarity occurs around the 16-cell stage in cattle [22] and after the 32-cell stage in rabbit embryos [22, 23]. Similar changes in microvillous distribution were noted in 10- to 18-cell compacting human embryos [24]. It is suggested, therefore, that timing of polarity development may coincide with embryo compaction and differs among species. Additionally, a study in mice [11] reported that a migration of cell nuclei from a central to basal location occurred before embryo compaction. However, to our knowledge, this interesting developmental event has not been reported in other species. The objectives of the present study were 1) to determine the timing of cell polarity development and 2) to document migration of embryonic cell nuclei in hamster embryos using scanning electron microscopy (SEM) and immunocytochemical staining methods.

MATERIALS AND METHODS

Collection of Embryos

Golden hamsters (*Mesocricetus auratus*), 10–12 wk old, were kept under conditions of constant temperature (21–23°C) and a cycle of 14L:10D (lights-on at 0500 h). They were given laboratory chow (MF; Oriental Yeast Co. Ltd., Tokyo, Japan) and water ad libitum. The estrous cycle of each female was determined according to Orsini [25]. In the evening of Day 4 of the estrous cycle, females were caged with males and checked for presence of spermatozoa in the vaginal smear. Successfully mated hamsters were sedated with ether and killed by cervical dislocation. Embryos at 1-cell to blastocyst stages were flushed from the oviducts and/or uteri with Dulbecco's PBS (DPBS; Gibco, Grand Island, NY) supplemented with 0.1% polyvinyl alcohol (PVA; Sigma, St. Louis, MO; DPBS-PVA). One-cell embryos were recovered at 1830–1930 h of Day 1, 2-cell embryos at 0530–0630 h of Day 2, 4-cell embryos at 0300–0500 h of Day 3, early 8-cell embryos at 1100–1200 h of Day 3, late 8-cell embryos at 1830–2000 h of Day 3, and morulae and blastocysts on Day 4. The zona pellucida (ZP) was removed with prewarmed, acidified DPBS-PVA (pH = 2.5) for 30–45 sec and 0.5% pronase for 1–2 min.

Decompaction and Disaggregation

Compact embryos were decompacted by incubation for 20 min in calcium-free DPBS. Embryos were disaggregated

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to single blastomeres by repeated pipetting with a flame-polished micropipette.

SEM Observations

Methods for preparing embryos and blastomeres for SEM observations have been reported previously [22]. Briefly, intact embryos and dissociated blastomeres were fixed for 1 h in 3% glutaraldehyde and 0.5% paraformaldehyde in Hanks' balanced salt solution (Gibco) with 0.1% polyvinyl alcohol (HBSS). They were washed in 3 changes of HBSS and placed on small glass coverslips (6 × 6 mm) coated with 0.1% poly-L-lysine solution (Sigma). Intact embryos or disassociated blastomeres on the coverslips were postfixed in 1% osmium tetroxide in HBSS for 1 h. After being rinsed, samples were incubated in 2% tannic acid solution for 2 h, rinsed, and then reosmicated for 1 h in 1% osmium tetroxide in distilled water. Specimens were then dehydrated in a series of increasing concentrations of ethanol, critical point-dried, and sputter-coated with gold. Observations were made with a JSM5300 scanning electron microscope (JEOL, Tokyo, Japan) at an accelerating voltage at 10 kV.

Fluorescent Observations

Labeling by fluorescein-isothiocyanate (FITC)-conjugated concanavalin A (Con A). FITC-conjugated Con A (FITC-Con A) was used to label the position of the microvillous pole on intact and isolated cells [2, 5, 11]. Blastomeres were fixed in 4% paraformaldehyde, washed, and incubated for 15 min at room temperature in FITC-Con A (100 μg/ml in PBS + 0.4% BSA + 0.02% sodium azide). Samples were examined using a fluorescence microscope (IMT2-RFC; Olympus, Tokyo, Japan) equipped with a DMIB filter set (Olympus) for FITC.

Labeling of nuclei and cytoskeleton. Preparation of the fixative was based on protocols of Albertini and Clark [26] and Aman and Parks [27] with minor modifications. Microtubule stabilization buffer (MTSB) was prepared as a stock solution (pH = 6.9) containing 0.5 M PIPES, 25 mM magnesium chloride, and 12.5 mM EGTA. Fixative was prepared by mixing 2 ml MTSB stock solution with 0.01% aprotinin, 1 mM dithiothreitol, 50% deuterium oxide, 1 μM paclitaxel, 5% Triton-X, and 5.4% formaldehyde, and made up to 10 ml with distilled water. Intact embryos or dissociated blastomeres were incubated in the fixative at 37°C for 1 h and then left overnight at 4°C before fluorescein staining.

The fixed cells were exposed to anti-β tubulin primary antibodies (1:200; Sigma) at 4°C overnight, then washed with wash solution containing 2% BSA, 2% goat serum, 0.2% milk powder, 0.2% sodium azide, and 0.1% Triton-X in calcium-free DPBS. The cells were subsequently incubated with the FITC-conjugated secondary antibody (1:200; Sigma) at 37°C for 2 h and then stained with rhodamine-phalloidin (1:1000; Molecular Probes, Eugene, OR) for microfilaments for 1 h and Hoechst 33342 (10 μg/ml) for DNA.

Samples were viewed on an Olympus microscope (BX-FLA; Olympus). A U-MWIB filter set (Olympus) was used for FITC, a U-WIB set (Olympus) for rhodamine, and a U-MWU set (Olympus) for Hoechst 33342. A cooled CCD video system (ImagePoint; Photometrics Ltd., Tucson, AZ) was used to obtain images on the personal computer, and color adjustment was performed by IPLab-Spectrum P software (Signal Analytics Corporation, Vienna, VA).

Data Analysis

Proportional data were analyzed by chi-square test.

RESULTS

SEM Observations

A total of 443 individual blastomeres from 128 hamster embryos before (1- to 7-cell stage), during (8-cell stage), or after (9- to 12-cell stage) compaction were processed for SEM. Pre-compacted embryos had relatively spherical blastomeres, whereas compacted embryos contained more flat blastomeres. Decompaction in calcium-free medium, however, made observations of microvilli distribution on the surface easier. SEM observations revealed that 1- to 7-cell-stage embryos as well as the majority of the early 8-cell embryos showed a dense and uniform distribution of microvilli over the cell surface (Fig. 1, a–c), although the distribution density of microvilli in the 8-cell embryos (Fig. 1c) decreased relative to younger embryos (Fig. 1, a and b). Ten percent of the blastomeres (n = 80) at the early 8-cell stage showed microvillous polarization (Table 1). Late into the 8-cell stage, polarized blastomeres were noted more frequently (28%, n = 128). In contrast to the mouse embryo, the microvillous pole in most polar blastomeres of the hamster embryo was found in the basal region of cell-cell contact, while fewer microvilli were present at the apical, outward-facing surface of the each blastomere (Fig. 1, d and f). Dissociation of blastomeres clearly allowed surface characteristics of the nonpolar (Fig. 1e) and polar cells (Fig. 1f) to be demonstrated. Mean population of polarized blastomeres increased at the late 8-cell stage (28%) and at the morula stage (35%). Some blastomeres (9–15%) at and after compaction were difficult to isolate, and their polarity could not be determined.

Labeling by FITC-Con A

Labeling patterns with FITC-Con A did not vary with the developmental stage of the embryos (data not shown). Both the intact embryos and the dissociated cells were surface-labeled with FITC-Con A irrespective of the cell stage; however, an accumulation of FITC label was frequently noted in that region of cytoplasm adjacent to another blastomere.

TABLE 1. Polarity of disaggregated blastomeres of hamster embryos evaluated by SEM.

Embryo stage	No. of embryos	Blastomeres evaluated	% Blastomeres (range)*		
			Nonpolar	Polar	Undetermined
1–7-cell	90	100	100	0	0
Early 8-cell[a]	10	80	81 (25–100)	10 (0–38)	9 (0–25)
Late 8-cell[b]	16	128	60 (12–100)	28 (0–88)	12 (0–50)
9–12-cell[b]	12	125	50 (11–56)	35 (20–78)	15 (0–40)

* Mean percentages (range); range shows minimum to maximum percentages of polar or nonpolar cells observed among embryos.
[a,b] Proportions in embryo stages with different superscripts differ significantly by chi-square test ($p < 0.01$).

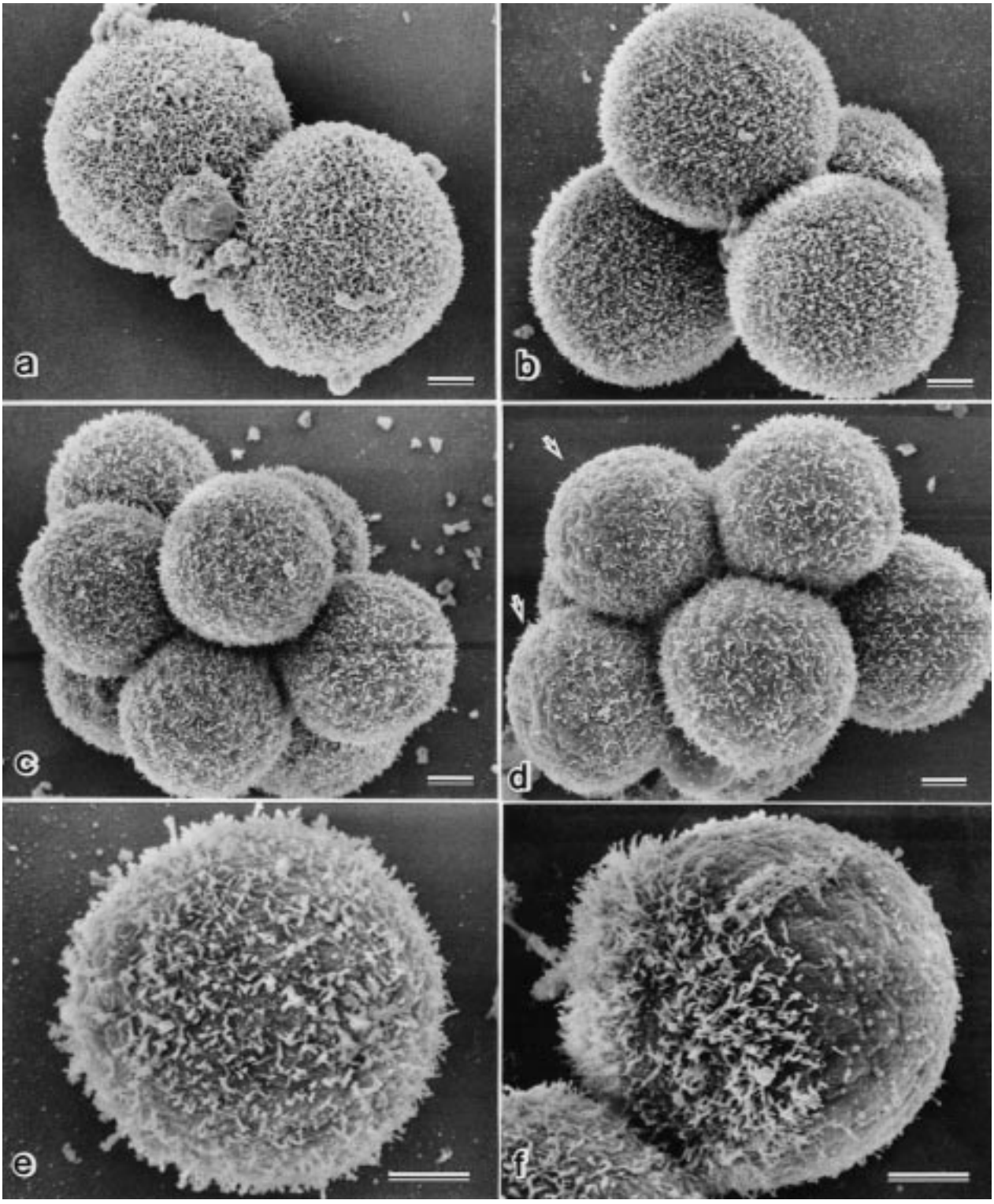

FIG. 1. Surface ultrastructure of hamster embryos examined by SEM. Bar represents 5 μm. **a**) An intact 2-cell embryo; **b**) 4-cell embryo; **c**) an early 8-cell embryo. Note a uniform distribution of microvilli for 2- to early 8-cell embryos and a decreased number of microvilli in the 8-cell embryo relative to 2- and 4-cell embryos. **d**) A late 8-cell embryo (decompacted in Ca-free DPBS). Notice polarized blastomeres (arrows), which show nonmicrovillous areas on the outside of the embryo and a microvillous pole appearing adjacent to regions of cell apposition. **e**) A nonpolar dissociated blastomere of a late 8-cell embryo; **f**) a polar blastomere of a late 8-cell embryo; notice a natural couplet (2/8) of a late 8-cell embryo showing microvillous pole located in the regions of basal, cell-cell contacts.

These features were coincident with the observation by SEM, which revealed that microvillous poles were generally faced inward and were situated directly between adjacent blastomeres in the hamster embryo, in contrast to those of mouse 8-cell embryos [2–8].

Labeling of Nuclei and Cytoskeleton

An outward migration of blastomere nuclei was observed with the progress of embryo development at compaction (Table 2). At 1- to 7-cell stages, all blastomere nu-

TABLE 2. Nuclear position in blastomeres at different developmental stages of hamster embryos.*

Embryo stage	No. embryos examined	% of blastomeres with nuclei (range)			
		At center	Migrating	Outward	Karyokinetic
1 cell	14	100	0	0	0
2 cell	27	98 ± 2 (50–100)	0	0 (0–50)	2 ± 2
4 cell	28	91 ± 3 (25–100)	0	0 (0–75)	8 ± 3
5–7 cell	18	85 ± 3 (57–100)	0	0 (0–43)	13 ± 3
Early 8 cell[a]	65	69 ± 6 (0–100)	20 ± 3 (0–75)	9 ± 2 (0–88)	3 ± 1 (0–25)
Late 8 cell[b]	49	9 ± 2 (0–75)	5 ± 1 (0–38)	80 ± 3 (13–100)	5 ± 2 (0–38)
Morula[c]	17	31 ± 4 (11–89)	22 ± 4 (0–45)	36 ± 5 (11–89)	11 ± 4 (0–50)

* Mean percentages ± SEM (range); range shows minimum to maximum percentages of blastomeres observed among embryos.
[a,b,c] Proportions in embryo stages with different superscripts differ significantly by chi-square test ($p < 0.01$).

clei were located in the center or near the center of the cell or occasionally in the basal half closer to the point of cell contact (Fig. 2, a and b). At the early 8-cell stage, most nuclei were similarly located in the center of the cell (Fig. 2, c–1), but some had begun to migrate outward, toward the cell surface (Fig. 2, c–2). By the late 8-cell-stage, 80% (n = 49) of blastomeres were found with their nuclei localized to the apical part of the cell (Fig. 2, d-1, d-2, and e). Such features were also noted in the outer blastomeres of morula-stage embryos (Fig. 2f).

Microtubules were present as a network throughout the cytoplasm during interphase (Fig. 2, a–f), whereas during karyokinesis they were seen only in the mitotic spindle around the two sets of chromosomes (Fig. 2a). Microfilaments, in contrast, were observed mainly just under the cell membrane and were more concentrated in the regions of cell-cell contact (Fig. 2, a–e). The intensity of microfilaments decreased dramatically at the noncontact regions of the cell surface during the second cleavage, and a continuous loss of microfilaments from these regions was apparent throughout subsequent embryo development (Fig. 2, a–f).

DISCUSSION

SEM of hamster 1- to 7-cell embryos and their dissociated blastomeres showed a uniform distribution of microvilli. Onset of polarization of microvillous distribution was noted at the early 8-cell stage. More polar cells were observed as compaction progressed (Table 1). Con-A staining of hamster embryos regardless of stages did not reveal any polarity of their cells in the present study. Previous studies in mice reported that binding sites of ligand, such as Con A and peanut agglutinin, may indicate the presence of microvilli at the heavily labeled pole [2–8]. However, we could not detect a polarity in FITC-Con A binding in these blastomeres, which displayed a ring stain irrespective of the embryo's developmental stage. Attempts to detect polarity using FITC-Con A were also unsuccessful for cattle and rabbit embryos [22], but limited success was achieved in cattle when laser confocal microscopy was employed [28]. We found in the present study that an accumulation of labeling was restricted to the internal surface membranes of blastomeres in the cleaved embryo. Some authors observed heavy labeling of FITC-Con A at the cleavage furrow of the mouse embryo [5]; probably this is the area associated with the actin filaments of the contractile ring. The reasons for the differences between our results in hamsters and previous studies in mice are not clear, although this difference can probably be attributed to the different mechanisms of embryo compaction among different species.

It has been reported that microvilli include a parallel linear array of microfilaments [1, 3]. In the present study, microfilaments were observed at a much higher density in the region of cell-cell contact than in the outward free cell surface. However, a strong FITC-Con A stain and higher density of microfilaments in this contact region may not always indicate a higher density of microvilli polarity, because we obtained similar results from pre- and post-compacted embryos by both fluorescence staining methods, but not by SEM as described above. Reeve and Ziomek [2] also reported discrepancies in the assessment of polarity by SEM and fluorescent labeling. They found that more than 30% of the blastomeres scored as nonpolar by FITC-Con A had microvillous poles under SEM. The actin staining results suggest that microfilaments may play an important role in cell-cell contact and cell flattening in the hamster, as has been shown in the mouse [29].

In the present study, the microvillous pole in the hamster embryos was found to be restricted to the basal region of cell-cell contact. In the mouse, however, the microvillous pole is located at the outer surface of the embryos [1–8]. In bovine embryos, the microvillous pole was not always oriented centrally on the apical or the basal regions [22]. The biological significance of the species difference in polarity orientation is not clear.

Another fascinating observation from the present study is that the nucleus of the embryonic blastomere in the hamster manifested a dramatic migration within the cytoplasm during early embryo development. From the early to the late 8-cell stage, hamster embryonic cell nuclei moved from the center to the peripheral, apical region of the cells. To our knowledge, this is the first report describing an outward migration of nuclei in embryos. In the mouse, Reeve and Kelly [11] reported that during the 4- and 8-cell stages the peripherally located nuclei became clustered nearer the center of the embryo. The direction of nuclear migration seems to be completely reversed in the hamster. Again, the biological significance of the nuclear outward migration and the species difference in the direction of embryonic cell nuclear migration is not known. However, it is apparent that the onset of the observed nuclear migration coincided with the timing of embryo compaction, an important physiological event during early embryo development that leads to subsequent embryonic cell differentiation. The migration of the embryonic cell nuclei is probably caused by rearrangement of cytoskeletal architecture during embryonic compaction. This proposition is supported by the observations that the nuclear position is found in the opposite pole of the polarized microvillous pole in both mice [11] and hamsters (this study). Furthermore, nuclear migration also coincided with the re-location of microtubules and microfilaments during embryo compaction (Fig. 2, d and

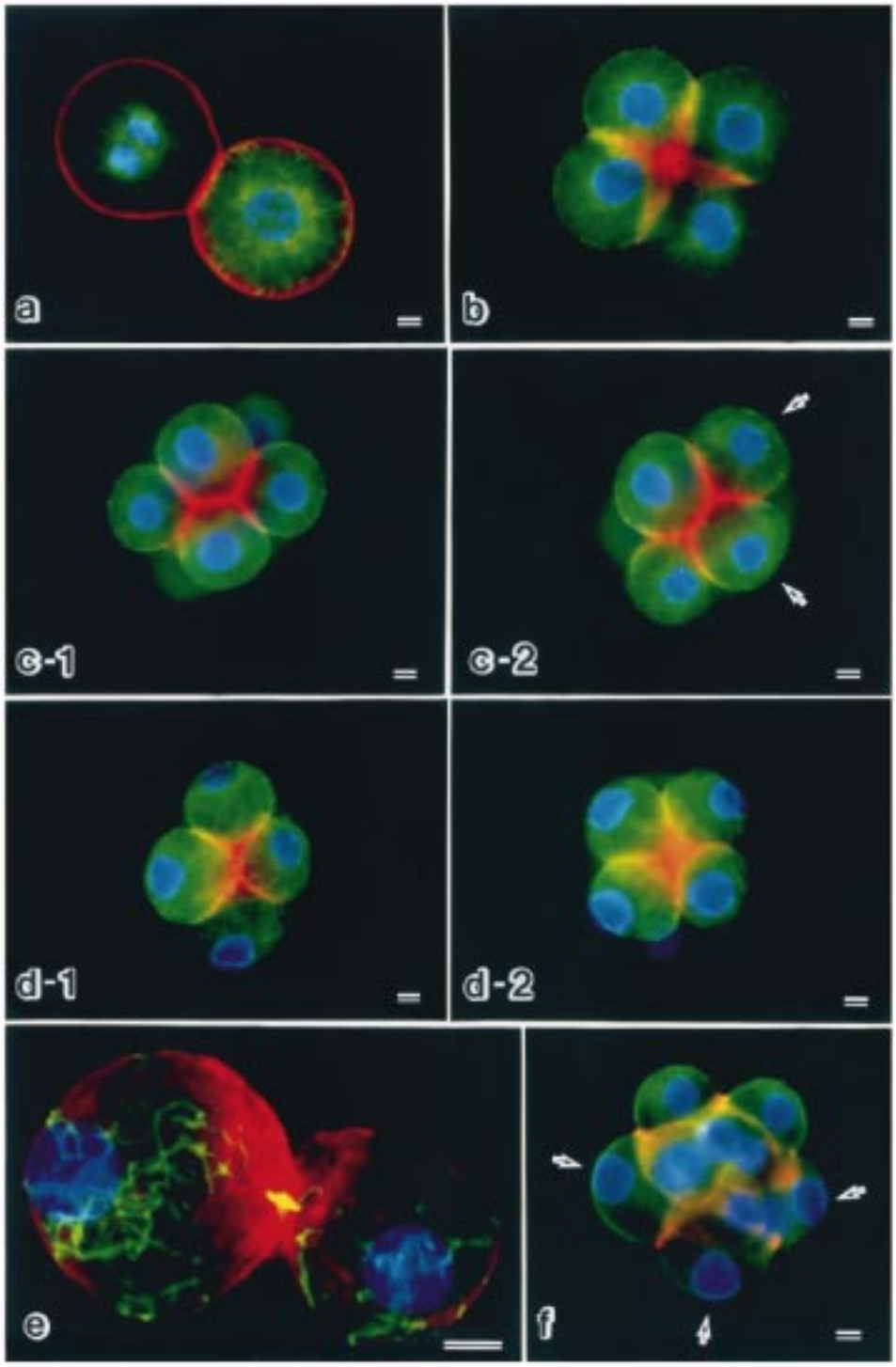

FIG. 2. Fluorescence photomicrographs of hamster embryos showing distribution of the nucleus and cytoskeleton. Bar represents 10 μm. Microtubules are green, microfilaments are red, and nuclei/chromosomes are blue; yellow shows the distribution of microtubules and microfilaments. **a**) A 2-cell embryo; notice that one blastomere is karyokinetic; **b**) a 4-cell embryo; **c-1**) 4 blastomeres from an early 8-cell embryo, **c-2**) the other 4 blastomeres from the same embryo; **d-1**) 4 blastomeres from a late 8-cell embryo, **d-2**) the other 4 blastomeres from the same embryo; **e**) a natural couplet from a late 8-cell embryo; **f**) a morula decompacted in Ca-free DPBS. Notice that the nuclei of individual cells are located in the center region of the cytoplasm of the 2-cell (**a**) and 4-cell (**b**) embryos, as are most nuclei of the early 8-cell embryo (**c-1**), and that some nuclei of the early 8-cell embryo are seen migrating to the outward side of the blastomeres (**c-2,** arrows), whereas all nuclei of the late 8-cell embryo are located in the apical region of the blastomeres (**d-1, d-2**). Also notice that the nucleus at the apical part of the blastomere seems to be supported by a network of microtubules (**d–f**) and that abundant microfilaments are seen in the region of cell-cell contact (**e**).

f). Additionally, it has been reported that the nucleus (including the germinal vesicle) is located in the microtubule domain of the cells in several species [30–32]. Polarity of actin distribution was obvious at compaction of the hamster embryos. It has been demonstrated, by analysis using embedment-free sections, that directional polarity of cytoskeletons occurred in cells from hamster embryos undergoing compaction [33]. In conclusion, embryonic cell polarity in hamsters became manifest during compaction at 8-cell stage; the microvillous pole appeared to be restricted to the basal cell-cell contact region; and an outward migration of the nucleus was observed for the first time in the hamster around the time of embryonic cell compaction.

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