Development 100, 685–698 (1987)
Printed in Great Britain © The Company of Biologists Limited 1987

Do trophectoderm and inner cell mass cells in the mouse blastocyst maintain discrete lineages?

JONATHAN DYCE, MARTIN GEORGE, HARRY GOODALL* and TOM P. FLEMING†

Department of Anatomy, University of Cambridge, Downing Street, Cambridge CB2 3DY, UK

* Present address: Hallam Medical Centre, 77 Hallam Street, London W1N 5LR, UK
† To whom all correspondence should be addressed

Summary

The extent to which trophectoderm (TE) and inner cell mass (ICM) lineages in the mouse blastocyst remain distinct during the period from the commencement of cavitation up until 48 h later in culture was investigated. Fluorescent latex microparticles were used to label exclusively all TE cells in nascent blastocysts and the position of labelled progeny in cultured blastocysts was examined by disaggregation, by serial sectioning and by whole-mount analyses. The results indicate that, in most blastocysts (80–90 %), TE and ICM lineages are entirely separate during this period while in the remainder lineage crossing is limited usually to only one or two cells of either tissue.

Key words: mouse blastocyst, trophectoderm, inner cell mass, cell lineage, fluorescent latex microparticles, horseradish peroxidase.

Introduction

At the blastocyst stage of mammalian development an outer polarized trophectoderm epithelium (TE) encloses the blastocoel and the eccentrically placed inner cell mass (ICM). These two tissues differ both in their morphology and physiology (Nadijcka & Hillman, 1974; Ducibella, Albertini, Anderson & Biggers, 1975; Borland, 1977; Handyside & Johnson, 1978; Johnson & Ziomek, 1982; Fleming, Warren, Chisholm & Johnson, 1984). They also differ in their prospective fates; thus, analyses of chimaeric post-implantation conceptuses derived from genotypically distinct TE and ICM cell populations have shown that most placental tissues (extraembryonic ectoderm, ectoplacental cone, trophoblastic giant cells) are of TE origin, while the ICM gives rise to the entire foetus and remaining extraembryonic membranes (reviewed in Gardner, 1978; Beddington, 1983; Rossant, 1984).

Cell lineage studies in early mammalian embryos have been confined largely to the mouse. These studies have been instructive with regard to the mechanisms of lineage derivation but have not defined clearly the time and stage of development when lineages become unambiguously separated. The TE phenotype is elaborated progressively in outer polarized blastomeres during cleavage from the 8- to 32-cell stage (Ducibella & Anderson, 1975; Ducibella, Ukena, Karnovsky & Anderson, 1977; Handyside, 1980; Ziomek & Johnson, 1980, 1982; Reeve, 1981; Reeve & Ziomek, 1981; Fleming & Pickering, 1985; Maro, Johnson, Pickering & Louvard, 1985; Fleming & Goodall, 1986; Fleming, Cannon & Pickering, 1986) at which time a permeability seal is formed and cavitation commences by virtue of vectorial fluid transport (Ducibella *et al.* 1975; Borland, 1977; Magnuson, Demsey & Stackpole, 1977). The ICM originates from an inward allocation of non-polar cells derived at division from the basal region of some polar 8- and 16-cell blastomeres (Johnson & Ziomek, 1981; Bałakier & Pedersen, 1982; Ziomek & Johnson, 1982; Ziomek, Johnson & Handyside, 1982; Johnson, 1986; Pedersen, Wu & Bałakier, 1986; Fleming, 1987). The occurrence of such 'differentiative' divisions, yielding polar and non-polar descendants, illustrates that, during the maturation of the TE lineages cell pluripotency is maintained and expressed within outside blastomeres up to at least the 16- to 32-cell transition. Whether, during blastocoel expansion, 32-cell-stage TE cells can also generate ICM derivatives by differentiative cleavage is less clear.

Earlier studies have shown that outside cells from late morulae and early blastocysts remain potent in this respect, but their precise cell stage was unknown (Stern & Wilson, 1972; Rossant & Vijh, 1980). In contrast, recent horseradish peroxidase (HRP) microinjection studies have demonstrated that nearly all injected presumptive 32-cell TE cells in early blastocysts gave rise only to TE descendants (Cruz & Pedersen, 1985; Pedersen *et al.* 1986). This result strongly implies a restriction in TE potency coinciding with the sixth cell cycle and blastocoel expansion.

In contrast to the TE lineage, cells of the ICM remain pluripotent beyond the period of blastocoel expansion. Non-polar 16-cell blastomeres and early ICM cells can polarize and transform into TE cells if manipulated to an outside position for a sufficient period (Johnson, Handyside & Braude, 1977; Handyside, 1978; Hogan & Tilly, 1978; Spindle, 1978; Johnson, 1979; Rossant & Lis, 1979; Ziomek *et al.* 1982; Ziomek & Johnson, 1982; Johnson & Ziomek, 1983; Fleming *et al.* 1984; Nichols & Gardner, 1984; Chisholm *et al.* 1985). It appears that ICM pluripotency is preserved up until at least the late 32- or early 64-cell stage (Chisholm *et al.* 1985). Despite having such a lability, inside cells in the intact embryo remain within the ICM lineage up to at least the early blastocyst stage (32- to 64-cells; Gearhart, Shaffer, Musser & Oster-Granite, 1982; Fleming, 1987). However, recent indirect evidence has suggested that in later blastocysts ICM cells may indeed become externalized and contribute to the TE lineage (Cruz & Pedersen, 1985). Most studies on reconstituted blastocysts do not lend support to this model and show little or no indication of ICM-derived cells contributing to the postimplantation trophoblast (Gardner, Papaioannou & Barton, 1973; Papaioannou, 1982; Rossant, Vijh, Siracusa & Chapman, 1983; Barton, Adams, Norris & Surani, 1985). However, an unusually high ICM contribution to the placenta has been noted for a minority of chimaeric conceptuses (Rossant & Croy, 1985).

In the present study, the cell lineage relationship between TE and ICM tissues has been examined in cultured blastocysts over a 48 h period from the time of initial cavitation. Fluorescent latex microparticles (Fleming & George, 1987; Fleming, 1987) have been used as an extrinsic marker to label exclusively the entire TE of nascent blastocysts. By analysing the distribution of labelled progeny within cultured blastocysts, we have been able to quantify the extent to which TE and ICM lineages remain discrete over this period. Our results indicate that, in most (80–90 %) embryos, lineages remain entirely distinct, while in the remainder usually one or two cells from either tissues are derived from the opposite lineage.

Materials and methods

Embryo collection, culture and staging

Female MF1 mice (Olac derived; Cambridge University Central Animal Services) were superovulated by intraperitoneal injections of 5 i.u. pregnant mare's serum and, 48 h later, 5 i.u. human chorionic gonadotrophin (hCG; Intervet) and mated overnight with HC-CFLP (Hacking & Churchill Ltd) males. A vaginal plug the following morning indicated successful mating.

Embryos were recovered at 67 h post-hCG injection (8-cell stage) by flushing oviducts with Hepes-buffered Medium 2 plus 4 mg ml^{-1} bovine serum albumin (M2 + BSA; Fulton & Whittingham, 1978). Embryos were cultured up until the onset of cavitation in Medium 16 plus 4 mg ml^{-1} BSA (M16 + BSA; Whittingham, 1971) under oil in Sterilin dishes at 37°C and 5 % CO_2 in air. During 72–80 h post-hCG (8- to 16-cell stage), the zona pellucida was removed by brief exposure to prewarmed acid Tyrode's solution (pH 2·5 + 4 mg ml^{-1} polyvinyl pyrollidone; Nicholson, Yanagimachi & Yanagimachi, 1975) followed by washes in M2 + BSA. Zona-free embryos were cultured individually in 10–15 μl drops of M16 + BSA.

From between 88–96 h post-hCG, stock zona-free embryos were examined hourly for the presence of a nascent blastocoelic cavity using a Wild dissecting microscope fitted with a heated (37°C) stage (Chisholm *et al.* 1985). Those embryos that had begun cavitation since the previous inspection were pooled (designated 0 h postcavitation blastocysts) and used for cell lineage labelling either immediately or after a further period in culture. All postcavitation embryos were cultured individually at 37°C and 5 % CO_2 in air in 25 μl drops Dulbecco's Modified Eagle's Medium supplemented with 10 % foetal calf serum (DMEM + FCS; Flow) under oil in Sterilin dishes to prevent attachment.

Cell lineage labelling

Zona-free blastocysts were incubated in a suspension of fluorescent latex microparticles (FL; yellow–green Fluoresbrite, 0·2 μm diameter, Polysciences) diluted 1:50 in M2 + BSA for 30–60 min at 37°C. By this procedure, all TE cells are labelled but transcytosis of FL into the blastocoel or adjacent ICM cells does not occur (Fleming & George, 1987). Following incubation, blastocysts were washed in M2 + BSA.

In some experiments, FL-labelled embryos at 2–3 h postcavitation were also labelled by ionophoretic microinjection of a combined solution of horseradish peroxidase (HRP; Sigma Type VI, 2·5 %) and rhodamine-conjugated dextran (RD; Sigma, 2 %) in 0·05 M-KCl. The injection procedure closely resembled those described previously (Goodall & Johnson, 1984; Goodall, 1986). Microelectrodes were pulled from 1·5 mm 'Kwik-Fil' tubing (Clarke Electromedical, Pangbourne, UK) and the tip filled with HRP + RD and the shaft with 0·05 M-KCl. Blastocysts for injection were maintained in M2 + BSA at 37°C on the water-heated stage of a Leitz Ortholux microscope and were held in position at the abembryonic pole by a suction holding pipette attached to a Leitz micromanipulator.

Injections took place in the central region of the polar trophectoderm (see Cruz & Pedersen, 1985), using 5 s pulses of a 5 nA depolarizing current with 1 s intervals for a total of 40 s. The pulse was applied using a Digitimer Neurolog System (Digitimer Ltd, Welwyn Garden City, UK) with the addition of a current monitor (Purves, 1981) to warn of blockage. During injection, a stable resting potential was recorded consistently. At 30–60 min after injection, blastocysts were examined under fluorescence for a very brief period (2–4 s) for the presence, position and number of RD-labelled cells before being returned to culture.

Cell lineage analyses

Three methods were used to analyse the distribution of FL-labelled cells within cultured blastocysts.

(a) *Disaggregation analysis*

Blastocysts were incubated in rhodamine-conjugated Concanavalin A (Rh-Con A, Polysciences) to label TE cells and subsequently disaggregated to small cell clusters in trypsin–EDTA as described previously (Fleming & George, 1987; Fleming, 1987) except that trypsin digestion was preceded by incubation in the microfilament-destabilizing drug, cytochalasin D (CCD; Sigma, $0{\cdot}5\,\mu g\,ml^{-1}$ in M2 + BSA) for 20 min at 37°C to facilitate opening of TE zonular tight junctions. Cells that were retained from each embryo were fixed for 30 min in individual drops of 4 % formaldehyde in phosphate-buffered saline (PBS), stored in M2 + BSA, transferred to wells of a tissue-typing slide (Baird & Tatlock) in M2 + BSA and examined for their fluorescent staining pattern.

(b) *Serial section analysis*

Intact blastocysts were fixed in 4 % formaldehyde + 0·5 % glutaraldehyde in PBS, buffer washed, dehydrated in an ethanol series and embedded in JB-4 water soluble resin (Polysciences) exactly as described previously (Fleming & George, 1987). Serial $3\,\mu m$ thick sections were viewed unstained for fluorescent labelling, using bright-field and phase-contrast microscopy to define cell outlines.

(c) *Whole-mount analysis*

This method was used for all embryos that were additionally labelled with HRP + RD, as well as some embryos that were labelled only with FL. Blastocysts were fixed in 4 % formaldehyde in phosphate buffer (0·1 M, pH 7·2, 20 min), washed in phosphate buffer and transferred to Tris–HCl buffer (0·05 M, pH 7·4) in which all subsequent processing was carried out. Embryos injected with HRP + RD were incubated in diaminobenzidine tetrahydrochloride (DAB; Sigma, $1\,mg\,ml^{-1}$) and 0·002 % fresh hydrogen peroxide for 10–20 min to visualize HRP-containing cells and then buffer washed. All blastocysts were incubated in $10\,\mu g\,ml^{-1}$ Hoechst dye for 20 min to label nuclei, buffer washed, briefly permeabilized in 0·25 % Triton X-100 for 30 s, buffer washed and incubated subsequently in rabbit anti-actin antibody (Miles; diluted 1:20, 40 min) and rhodamine-conjugated anti-rabbit immunoglobulin antibody (Miles; diluted 1:150, 40 min) to label cell outlines more clearly. Embryos were placed in individual $10\,\mu l$ drops of buffer under oil on a Falcon dish for microscopical examination using long-range objectives. A finely drawn microinjection needle attached to a Leitz micromanipulator was used to gently roll embryos so that all surfaces could be viewed *en face*.

Microscopy

A Zeiss Universal or a Leitz Ortholux II photomicroscope equipped with the appropriate filter sets for FL (487709 or L2), RD and rhodamine-actin labelling (487715 or N2), and Hoechst staining (A) was used for examining intact embryos, cells and sections. Photographs were taken on Kodak Tri-X 35 mm film.

Results

Embryo viability

The influence of FL labelling on preimplantation development has been examined in detail previously (Fleming & George, 1987). No effect was evident on the timing and expression of morphological changes during cleavage, early blastocyst cell number or the capacity of blastocyst tissues to differentiate further when compared with control unlabelled embryos. In the present study, where FL labelling took place at the onset of cavitation or later, apparently normal blastocyst expansion was observed in 92–100 % embryos during 48 h culture. Blastocyst cell number increased steadily during culture and was comparable with data from unlabelled embryos (see Table 1).

Disaggregation analysis

In these experiments blastocysts were either (a) labelled with FL at 0 h postcavitation and cultured for 2, 6, 12, 24 or 36 h or (b) labelled at 12 or 24 h postcavitation and cultured for a further 12 h. Following culture, blastocysts were Rh-Con A labelled to mark TE cells before disaggregation and analysis of labelling patterns (Table 2). In contrast to an earlier study (Fleming, 1987) where most or all cells per embryo from late morulae and nascent blastocysts could be retained during disaggregation, in the present experiments with more mature blastocysts, this figure was substantially lower (42, 33 and 72 % at 12, 24 and 36 h postcavitation respectively, based on estimates of total cell number from the serial sectioning analysis).

Four labelling patterns were observed. (1) Cells positive for Rh-Con A and FL corresponding to TE cells derived from TE progenitors in the nascent blastocyst. (2) Fluorescent-negative cells categorized as ICM cells originating from ICM progenitors. (3) Cells labelled only with Rh-Con A corresponding to TE cells descended from the early ICM. (4) Cells labelled only with FL, classified as ICM cells derived from the nascent TE layer (see Fig. 1). Since not all

Table 1. *Mean cell numbers within FL-labelled blastocysts cultured for up to 36 h postcavitation and, at 24 h postcavitation, compared with unlabelled blastocysts. Data from serial sectioning (summarized from Table 3) and from a modification of the DAPI technique (Chisholm* et al. *1985)*

Treatment (labelling time; h postcavitation)	Blastocyst age at analysis (h postcavitation)	Technique	Number analysed	Mean cell number ± S.D.*		
				Total	TE	ICM
FL labelled (0)	12	sections	5	44·2 ± 1·1	29·4 ± 4·1	14·8 ± 4·5
FL labelled (12)	24	sections	7	58·6 ± 8·4	40·4 ± 5·1	18·1 ± 4·6
FL labelled (0)	24	DAPI	14	65·7 ± 11·1	49·3 ± 8·0	15·6 ± 3·2
Unlabelled (0)	24	DAPI	14	60·7 ± 8·5	47·5 ± 8·6	15·6 ± 2·6
FL labelled (24)	36	sections	4	87·8 ± 5·6	59·5 ± 2·9	28·3 ± 2·8
FL labelled (0)	48	sections	2	109	83	26

* Standard deviation.

cells have necessarily undergone division from the time of FL labelling (e.g. short-duration cultures), the labelling pattern may also be interpreted as an undivided blastomere that has maintained position within the TE (1) or ICM (2), or has changed position (3, 4).

The results in Table 2 indicate that, in general, TE and ICM cell lineages remain discrete during the 36 h culture period. Of the 6729 cells analysed at different time points, only 59 (0·9 %) were derived from lineage crossing (see Fig. 1D–L). This proportion is slightly higher for ICM cells (1 %) than for TE cells (0·8 %). Moreover, of those embryos in which lineage crossing was detected (38 out of 238), in the majority (22; 58 %) this feature was observed in only one scored cell. In only one embryo were more than three such cells detected (Fig. 1J–L). Table 2 (part B) shows that the limited degree of lineage crossing detected is not restricted to a particular time postcavitation.

Serial section analysis

This method of analysis provided a more detailed picture of lineage relationships since *all* cells in an embryo could be examined and the spatial pattern of lineage interactions could be observed. In these experiments, the time period between FL labelling and fixation was either 0–48 h postcavitation or 12 h periods within this range (Table 3). In agreement with the disaggregation analysis, the results show that in general TE and ICM cell lineages remain completely separate during this period (see Fig. 2A,B). Collectively, only 11 out of 1288 cells (0·85 %) that were examined individually for their labelling characteristics were derived from the opposite lineage (0·8 % in TE, 0·9 % in ICM; these figures are an overestimate since cell numbers were not determined in

Table 2. *Fluorescent labelling patterns of cells disaggregated from FL-labelled blastocysts at various times postcavitation*

Time between FL labelling and disaggregation (h post-cavitation)	Number of embryos analysed	Total cell number analysed	Trophectoderm (TE) (Rh-Con A positive)		% TE cells of ICM origin	% embryos with 1 or more TE cells of ICM origin	Inner cell mass (ICM) (Rh-Con A negative)		% ICM cells of TE origin	% embryos with 1 or more ICM cells of TE origin
			FL positive	FL negative			FL positive	FL negative		
A 0–2	26	700	420	0	0	0	2 [2(1)]*	278	0·7	3·8
0–6	18	352	220	9 [1(2), 7(1)]*	3·9	16·7	1 [1(1)]	122	0·8	5·5
0–12	18	337	218	1 [1(1)]	0·4	5·5	3 [1(3)]	115	2·5	16·7
0–24	40	783	510	8 [1(6), 2(1)]	1·5	17·5	8 [1(2), 2(3)]	257	3·0	12·5
0–36	34	2143	1395	8 [1(3), 2(1), 3(1)]	0·6	14·7	6 [1(3), 3(1)]	734	0·8	11·8
B 12–24	63	1328	822	9 [1(1), 2(4)]	1·0	7·9	2 [1(2)]	495	0·4	3·2
24–36	39	1086	780	1 [1(1)]	0·1	2·5	1 [1(1)]	304	0·3	2·5

* Denotes the number of scored TE or ICM cells per embryo derived by lineage crossing (outer bracket) and the number of embryos showing this incidence (inner bracket), e.g. [1(2), 7(1)], 1 lineage-crossed cell in each of 2 embryos and 7 lineage-crossed cells in 1 embryo.

certain embryos where both lineages bred true, see Table 3 embryos 13–17 and 24–30). Of thirty embryos examined, four (13 %) exhibited a TE of heterogeneous origin (see Fig. 2C,D) and two (7 %) an ICM with TE-derived cells (see Fig. 2E,F); one embryo contained both TE and ICM cells of mixed origin. In all such embryos, cell lineage crossing was restricted to a maximum of two cells per tissue. TE

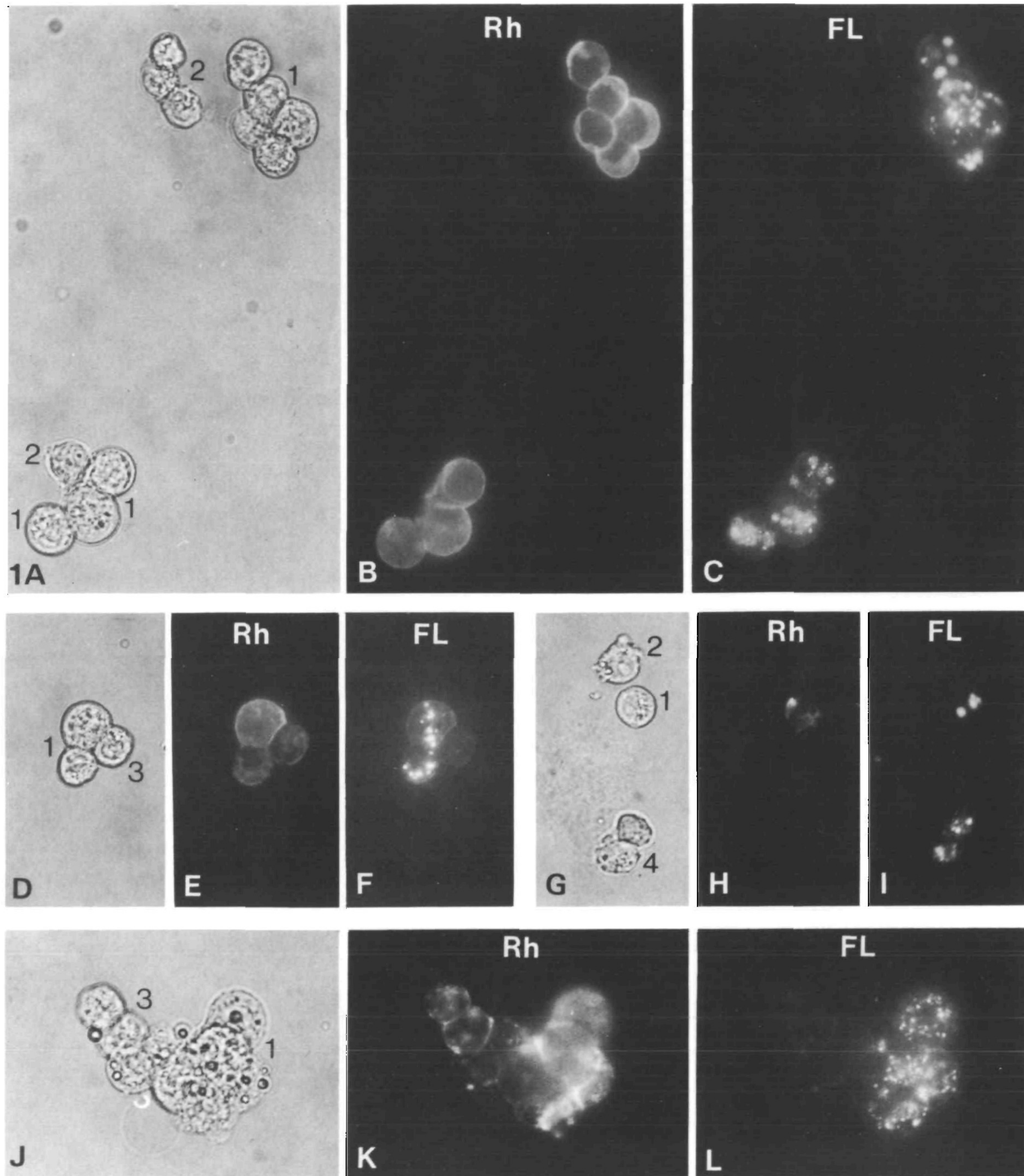

Fig. 1. Disaggregation analysis. Examples of fluorescent labelling patterns within cells derived from four blastocysts that were FL labelled at 0 h postcavitation and cultured for 36 h (A–C, D–F, G–I) or 6 h (J–L) before Rh-Con A staining and disaggregation. A,D,G,J, bright field; B,E,H,K, Rh-Con A fluorescence (Rh); C,F,I,L, FL fluorescence. (A–C) All cells are either labelled with both markers (category 1, see text) or are negative for both markers (category 2). (D–F) Two cells belong to category 1 while one cell is labelled only with Rh-Con A (category 3). (G–I) Category 1 and 2 cells are shown at the top while two cells labelled with FL only (category 4) are present below. (J–L) Cell cluster with all cells labelled with Rh-Con A (= trophectoderm) and most with FL (category 1); five cells are FL negative (category 3). ×300.

Table 3. *Serial section analysis of blastocysts labelled with FL and cultured for various times postcavitation*

Time between FL labelling and fixation (h post-cavitation)	Embryo number	Total cell number	Trophectoderm: FL positive	Trophectoderm: FL negative (position)†	Trophectoderm: Total	Trophectoderm: % cells of ICM origin	Inner cell mass: FL positive (position)†	Inner cell mass: FL negative	Inner cell mass: Total	Inner cell mass: % cells of TE origin
0–12	1	43	31	2(M)	33		0	10	10	
	2	43	28	0	28		0	15	15	
	3	45	24	0	24	2·0	2(AP)	19	21	2·7
	4	45	33	1(M)	34		0	11	11	
	5	45	28	0	28		0	17	17	
12–24	6	43	32	2(P)	34		2(AP)	7	9	
	7	53	34	0	34		0	19	19	
	8	57	40	0	40		0	17	17	
	9	61	42	0	42	1·4	0	19	19	
	10	63	41	0	41		0	22	22	
	11	66	46	2(P)	48		0	18	18	
	12	67	44	0	44		0	23	23	0·9
	13*	—	all	0	—		0	17	17	
	14*	—	all	0	—		0	17	17	
	15*	—	all	0	—		0	27	27	
	16*	—	all	0	—		0	13	13	
	17*	—	all	0	—		0	14	14	
24–36	18	81	56	0	56		0	25	25	
	19	86	59	0	59	0	0	27	27	0
	20	90	60	0	60		0	30	30	
	21	94	63	0	63		0	31	31	
0–48	22	110	75	0	75	0	0	25	25	0
	23	118	91	0	91		0	27	27	
	24–30*	—	all	0	—		0	all	—	

* These blastocysts were examined principally for cell lineage crossing. Cell numbers in TE or the complete embryo were not determined.

† P, within the polar TE; M, within the mural TE; AP, adjacent to the polar TE.

cells derived from the ICM were located either within the polar TE or close by in the adjacent region of mural TE (Fig. 2C,D). All ICM cells derived from TE were positioned in contact with the polar TE at least on one face (Fig. 2E,F).

Whole mount analysis

(a) *Embryos labelled only with FL*

Ninety eight embryos were labelled with FL at 0 h postcavitation and were cultured for 24 h ($n = 54$) or 48 h ($n = 44$) before whole-mount analysis. By this method only cellular contributions from the ICM (FL negative) to the TE could be detected. Hoechst staining and rhodamine-conjugated anti-actin staining were used to identify more clearly cell nuclei and intercellular boundaries in the TE (see Fig. 3). For polar TE analysis, actin staining was necessary since cell boundaries are not evident by bright-field microscopy and Hoechst staining resulted in interference from ICM nuclei. The results are summarized in Table 4. For both culture times, in 89 % blastocysts all TE cells were FL labelled (Fig. 3) while in remaining embryos either one or two TE cells were found to be FL negative (see Fig. 4 for examples of this in microinjected whole mounts). Unlabelled cells were located in the polar TE in all cases.

(b) *Embryos labelled with FL and microinjected with HRP + RD*

143 FL-labelled embryos were used for microinjection of HRP + RD into the polar TE at 2–3 h postcavitation (Fig. 4A,B). Of these, 86 (61 %) showed definitive RD fluorescence in the TE when viewed within 1 h postinjection [breakdown: 58 embryos, one cell labelled (67 % RD-labelled embryos); 27 embryos, two cells labelled (31 %); 1 embryo, three cells labelled (1 %)]. Remaining embryos were either RD-negative ($n = 54$) or contained RD in the blastocoele or an ICM cell ($n = 3$); these were discarded. RD-labelled embryos were either cultured for 24 h ($n = 14$) or 48 h ($n = 58$), or were fixed immediately and DAB-reacted to correlate RD and HRP labelling patterns ($n = 14$). Of these control embryos, 12

showed identical RD- and HRP-labelling for the number and position of injected cells; in 2 embryos, RD was visible in one TE cell and HRP in this plus an adjacent TE cell. RD fluorescence was therefore considered a reasonable but not a definitive guide to the number/position of TE cells receiving HRP at the time of microinjection. Most cultured embryos underwent apparently normal expansion and TE proliferation although 10 (14 %) at 48 h postcavitation were judged to have grown insufficiently or had degenerated and were discarded. Remaining cultured embryos ($n = 62$) were fixed and processed for whole-mount analysis of which 42 were scored successfully [embryos were discarded if damaged during processing ($n = 9$), cell labelling patterns were ambiguous ($n = 5$), HRP was present in the blastocoele ($n = 4$), HRP was present in an ICM cell ($n = 1$) or if HRP staining was not apparent ($n = 1$)].

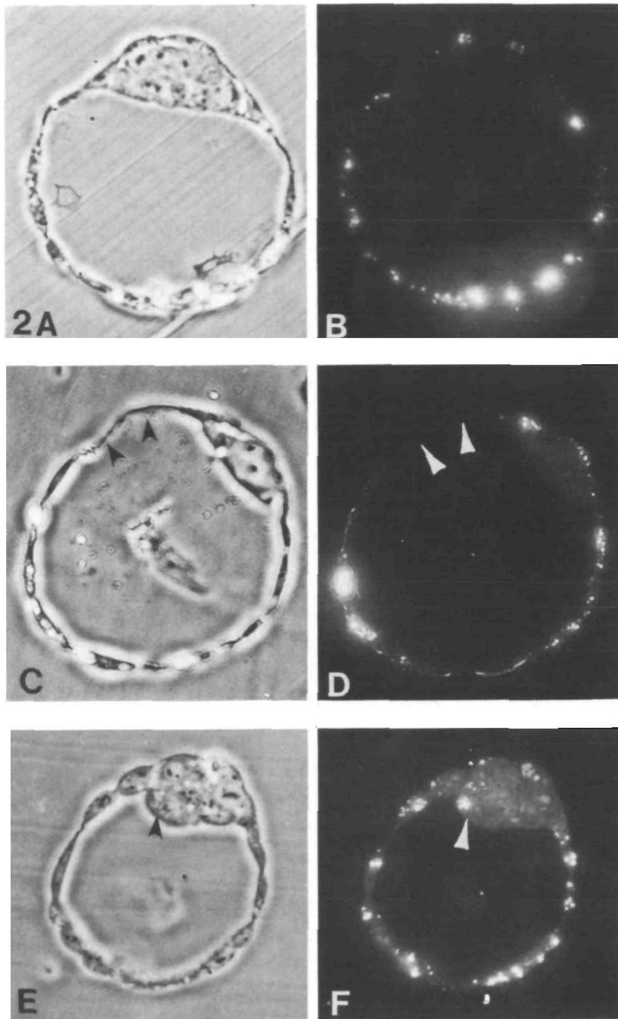

Fig. 2. The distribution of FL-labelled cells in blastocysts analysed by serial sectioning. Embryos were FL labelled at 12 h and analysed at 24 h postcavitation (A,B; E,F) or FL labelled at 0 h and analysed at 12 h postcavitation (C,D). (A,B) FL labelling is confined to all TE cells (embryo 7 in Table 3). (C,D) In this section, one cell in the mural TE close to the polar zone (arrowheads) is FL negative (embryo 1 in Table 3). (E,F) In this section, one ICM cell adjacent to the polar TE and blastocoel (arrowhead) is FL labelled (embryo 6 in Table 3). ×300.

The results for the limited number of successfully processed embryos are summarized in Table 5. Embryos cultured for 24 h showed only a small increase from the time of injection in the mean number of cells labelled with HRP + RD (Table 5A); one round of cell division of labelled cell(s) occurred in only 25 % embryos. After 48 h culture, the mean number of HRP-labelled cells per embryo was twice that at 24 h and in most embryos (74 %) at least one round of division of HRP + RD-labelled cells had taken place; in some embryos, three (9 %) or four (3 %) rounds of division had occurred (Table 5A). Thus, HRP + RD injection did not prevent, but may have delayed, subsequent division of labelled cells (in agreement with data from Cruz & Pedersen, 1985).

The position of HRP + RD labelled cells relative to the blastocyst embryonic: abembryonic axis appeared to change during culture. Prior to culture, most labelled cells (assessed by RD fluorescence) were in the central zone of the polar TE (Fig. 4A,B; Table 5B). Both at 24 h and at 48 h culture, the mean position of labelled progeny in most embryos had shifted towards the abembryonic pole (see Fig. 4C,D,H). After 48 h culture, HRP-labelled cells were located in the polar TE (32 % of total; $n = 112$), at the junction between polar and mural zones (29 %) and in the mural TE (38 %). The central polar TE cell was positive for HRP in only 26 % of all embryos (Table 5B). The displacement of HRP-labelled cells supports the previous data from Copp (1979) and

Table 4. *Whole-mount analysis of blastocysts labelled with FL at 0 h postcavitation and cultured for 24 or 48 h*

Incubation time (h)	No. embryos analysed	FL labelling pattern in trophectoderm. No. embryos (% of total) with		
		all cells labelled	all but one cell labelled	all but two cells labelled
24	54*	48 (89 %)	5 (9 %)	1 (2 %)
48	44*	39 (89 %)	2 (4 %)	3 (7 %)

* An additional 4 (24 h) or 7 (48 h) embryos were not analysed due to ambiguous FL labelling in relation to trophectoderm cell outlines.

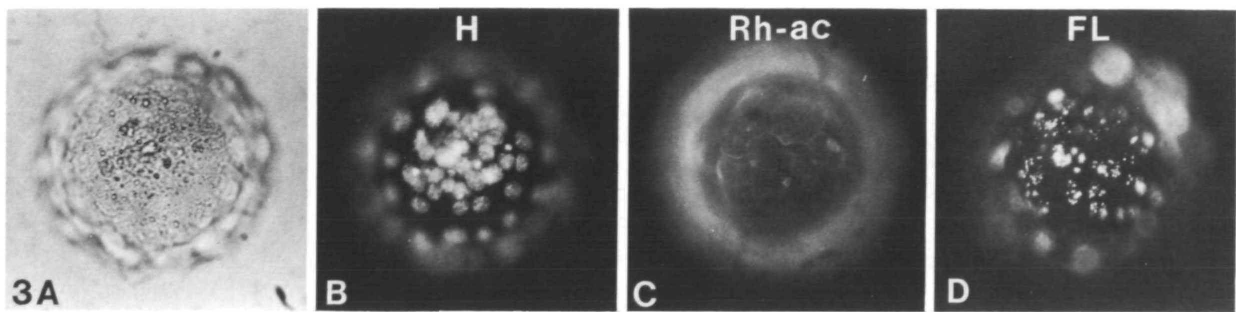

Fig. 3. Whole-mount analysis of blastocyst, FL labelled at 0 h postcavitation and cultured for 12 h before fixation. (A) Bright-field micrograph of the polar TE viewed *en face*. Cell outlines are not visible. (B) Hoechst staining showing aggregate of ICM nuclei. (C) Rhodamine–actin staining showing cell outlines in the polar TE. (D) FL labelling; all cells are labelled. ×300.

Cruz & Pedersen (1985) indicating that proliferating polar TE cells contribute descendants to the mural TE.

Analysis of FL-labelling in cultured embryos was consistent with the data presented in Table 4 and showed that in most embryos (79 %) all TE cells were FL-labelled, including the central zone of the embryonic pole (Fig. 4C–G,L–O; Table 5C). However, it was found that HRP-labelled cells were consistently weakly stained with FL (see Fig. 4G,K). Since these cells were derived from FL-labelled nascent polar TE cells, the low FL staining is artifactual and presumably caused by interference from HRP reaction product; all such cells were classified as FL positive. In remaining embryos, evidence for an ICM contribution to the TE was limited to the presence of no more than two FL-negative cells, usually, but not exclusively, located in the polar TE (Fig. 4H–K, P–S).

Polar trophectoderm injury

To examine whether exchange of cells between lineages could be increased by embryonic injury, the central polar TE cell of blastocysts labelled with FL at 0 h postcavitation was lesioned deliberately with a microinjection needle at 2 h postcavitation (Fig. 5), before further culture for up to 36 h and analysis. Control embryos were manipulated in an identical way but were not lesioned. To demonstrate that the lesioning procedure was effective and localized, 16 embryos were stained with 0·25 % trypan blue (to label lysed cells) immediately after treatment; one stained cell was observed in the polar TE in 15 embryos and two in the remainder.

The results from disaggregation experiments are shown in Table 6. Although lesioned embryos at both 24 and 36 h postcavitation possessed a slightly higher incidence of TE cells derived from the ICM than did controls, the difference is not significant and in the majority of lesioned embryos (29/34, 85 %) all TE cells examined were FL-labelled. Similarly, the contribution of TE cells to the ICM was not greater in lesioned embryos than in controls.

12 lesioned and 12 control blastocysts were also examined by serial sectioning either at 24 or 36 h postcavitation. The incidence of lineage crossing in both groups was similar and comparable to that presented in Table 3 (data not shown).

Fig. 4. Whole mounts of blastocysts FL labelled at 0 h and HRP+RD injected at 2–3 h postcavitation. (A,B) Blastocyst examined for position of RD-labelled cell(s) shortly after injection. Here, two cells in the central polar TE are labelled. Remaining panels (C–S). Four blastocysts (C–G, H–K, L–O, P–S) cultured up to 48 h postcavitation before analysis. C,D,H,L,P, bright field; E,I,M,Q, Hoechst nuclear staining; F,J,N,R, Rhodamine-actin staining; G,K,O,S, FL labelling. (C) Blastocyst orientated to show position of ICM in relation to HRP-labelled cells (arrow). Note absence of HRP in polar TE cell overlying ICM (arrowhead). (D) Blastocyst repositioned to view polar TE *en face* (corresponding with E–G). Three HRP-labelled cells partially encircling the centre of the polar TE are shown. Cell outlines in this region are not visible. (E) Note cluster of nuclei (arrowhead) corresponding to position of ICM. Nuclei in HRP-labelled cells are not labelled. (F) Outline of central polar TE cell revealed by actin staining. (G) FL labelling present in central cell. Note reduced FL staining in HRP-labelled cells. (H–K) Blastocyst positioned to view polar TE *en face*. A single HRP-labelled cell is seen at the bottom of the bright-field micrograph (H), below the cluster of nuclei corresponding to the ICM (I). Two cells in the central polar TE indicated by actin staining (arrowheads, J) are unlabelled with FL (K). (L–O) Blastocyst positioned to view region of mural TE. All cells shown are FL positive with the microparticles localizing in a ring around the nucleus. (P–S) Mural TE region of blastocyst in which two cells (arrowheads, R), one binucleate, are unlabelled with FL. A,B, ×185; C–S, ×300.

Discussion

The three methods used to analyse FL-labelled blastocysts gave results that were consistent and indicated that (a) in most embryos (80–90 %) TE and ICM lineages remain completely separate from the time of cavitation onwards, (b) in the remaining embryos cell lineage crossing is usually limited to no

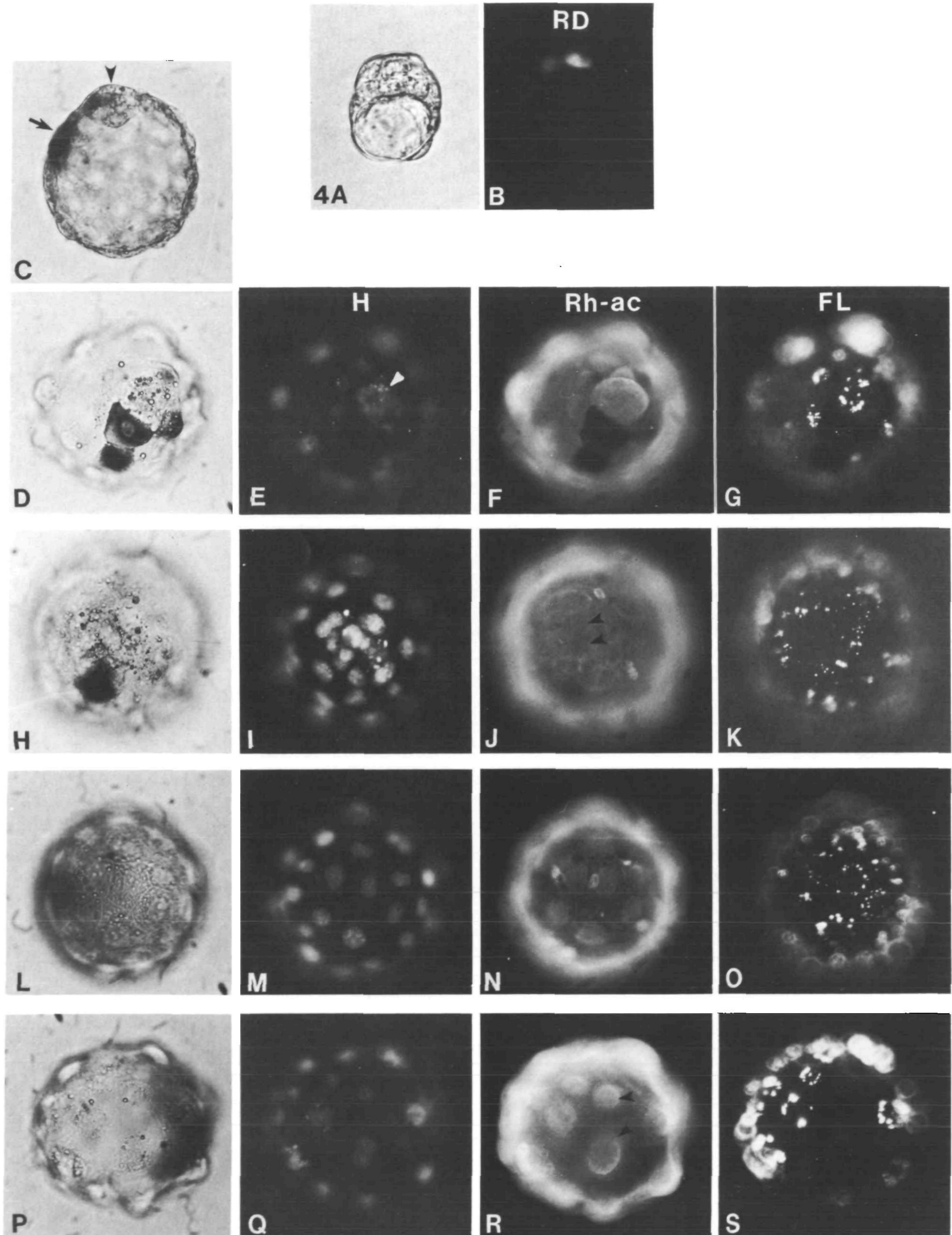

Table 5. *Whole-mount analysis of blastocysts labelled with FL at 0 h postcavitation, injected with HRP+RD in the polar TE at 2–3 h postcavitation and cultured for 24 or 48 h*

(A) *Division of injected cells*

Incubation time (number of embryos analysed)	Mean number of RD-labelled cells at time of injection ± S.D.	Mean number of HRP-labelled cells at time of analysis ± S.D.	Number of embryos (% of total) with estimated minimum number of cell divisions of HRP + RD injected cells being				
			0	1	2	3	4
24 h (8)	1·125 ± 0·35	1·625 ± 1·06 range 1–4	6 (75 %)	2 (25 %)	0	0	0
48 h (34)	1·235 ± 0·07	3·265 ± 2·45 range 1–12	9 (26 %)	12 (35 %)	9 (26 %)	3 (9 %)	1 (3 %)

(B) *Position of injected cells and their progeny**

	Position of RD-labelled cell(s) at time of injection	Number of embryos (% of total) with mean position (degrees from embryonic: abembryonic axis) of HRP-labelled cells at time of analysis being				Number of embryos (% of total) with central polar TE cell labelled with HRP
		0°	15–30°	45–60°	75°+	
24 h (8)	Central polar TE zone 8	0	1 (12·5 %)	4 (50 %)	3 (37·5 %)	0 (0 %)
48 h (34)	Central polar TE zone 28	2 (7 %)	6 (21 %)	11 (39 %)	9 (32 %)	11 (32 %)
	Off-central polar TE 6	1 (17 %)	1 (17 %)	3 (50 %)	1 (17 %)	

(C) *FL labelling pattern in trophectoderm*

	Number of embryos (% of total) with		
	all cells labelled	all but one cell labelled	all but two cells labelled
24 h (8)	7 (87·5 %)	1 (12·5 %)	0
48 h (34)	25 (79 %)	4 (12 %)	3 (9 %)

* Position of labelled cells determined by embryo rotation.

more than one or two cells and (c) when such a contribution occurs, the direction of expression can be either from ICM to TE or *vice versa* with similar probability. The delayed labelling experiments showed that when lineage crossing occurred it was not restricted to the early period following cavitation. Since nascent blastocysts (0–2 h postcavitation) are composed of approximately 32 cells (Chisholm *et al.* 1985; Fleming & George, 1987), it follows that cell lineages *in situ* are by and large segregated once cells enter the sixth cell cycle.

For TE cells, this conclusion is consistent with the earlier findings of Cruz & Pedersen (1985) and Pedersen *et al.* (1986) following HRP-injection studies. Although some of the rare examples of FL-labelled cells within the ICM may have derived from retarded (5th cell cycle) outside blastomeres, such cells were also found in embryos labelled after expansion (see Tables 2, 3). Thus, the restriction to the TE lineage observed in the intact blastocyst may not necessarily represent true commitment, but rather a physical constraint on the ability of cells *in situ* to divide differentiatively due to their lateral stretching during fluid accumulation and the maturation of intercellular junctions. Cell shape has already been shown to influence the division plane orientation of TE progenitors (polar blastomeres) during cleavage (Johnson & Ziomek, 1983).

The establishment of a developmentally restricted ICM lineage from early cavitation in most embryos

Table 6. *Fluorescent labelling patterns of cells disaggregated from 24 and 36 h postcavitation blastocysts following FL labelling at 0 h and lesion of the central polar TE cell at 2 h postcavitation*

Blastocyst stage (h post-cavitation)	Treatment	Number of embryos analysed	Total cell number analysed	Trophectoderm (TE) (Rh-Con A positive) FL positive	FL negative	% TE cells of ICM origin	Inner cell mass (ICM) (Rh-Con A negative) FL positive	FL negative	% ICM cells of TE origin
24	Lesioned	17	268	184	4 [4(1)]*	2·1	3 [1(1), 2(1)]*	77	3·8
	Control	18	412	312	0	0	11 [1(3), 2(1), 3(2)]	89	11·0
36	Lesioned	17	490	370	11 [1(2), 3(1), 6(1)]	2·9	5 [1(1), 2(2)]	104	4·6
	Control	25	641	517	4 [2(2)]	0·8	5 [1(5)]	115	4·2

* For explanation, see Table 2.

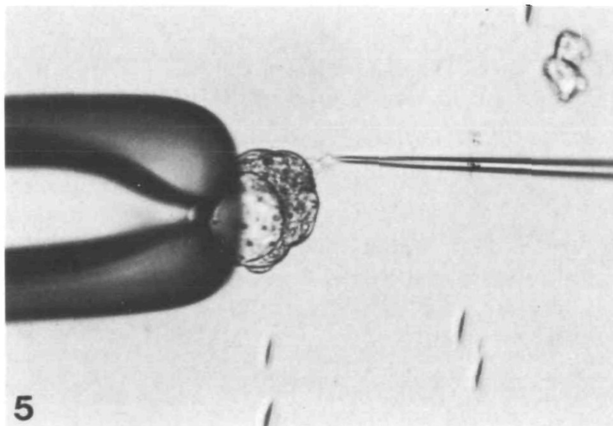

Fig. 5. Blastocyst labelled with FL at 0 h postcavitation and manipulated to lesion the central polar TE cell at 2 h postcavitation. The embryo is held by suction pipette while a microinjection needle is used to destroy the cell. ×150.

certainly precedes the time at which these cells become committed to an ICM fate (see Introduction). In fact, we have shown previously (Fleming, 1987) that the immediate fate of the earliest allocation of cells to the inside of the embryo (at the 8- to 16-cell transition) is nearly always to contribute exclusively to the ICM of nascent blastocysts. Taken together, these results indicate that once cells enter the prospective ICM lineage (by differentiative division of polar 8- or 16-cell blastomeres), this fate is realized with only very few exceptions. The importance of the adhesive envelopmental properties of polar cells and TE cells in maintaining the position of inside cells and ICM cells during early development has been discussed previously (Ziomek & Johnson, 1981, 1982; Kimber, Surani & Barton, 1982; Johnson & Ziomek, 1983; Surani & Handyside, 1983; Fleming *et al.* 1984; Surani & Barton, 1984; Fleming, 1987).

Our failure to detect a consistent and appreciable cellular contribution from the ICM to the polar TE during blastocyst culture is incompatible with the lineage model recently proposed by Cruz & Pedersen (1985). They labelled the central polar TE cell in midstage blastocysts by HRP injection and showed that, after up to 48 h culture, the labelled progeny were displaced towards the mural TE while the central zone of the polar TE was usually unlabelled. The displacement of HRP-labelled cells supported the earlier finding by Copp (1979) that increases in the mural TE cell population were derived from proliferation within the polar TE. To explain the absence of HRP in the central zone of the polar TE after culture, Cruz & Pedersen (1985) proposed that developmentally labile ICM cells were recruited into the polar TE and acted as a source of proliferation. We have performed a limited series of HRP-injection experiments similar to those of Cruz & Pedersen (1985) but on blastocysts additionally labelled with FL. The pattern of HRP labelling after culture was consistent with the earlier study and showed both the proliferation and displacement of HRP-labelled TE cells towards the mural zone and, in the majority of embryos, the absence of HRP labelling in the central polar TE. However, in agreement with our other analyses, the polar TE only rarely contained any FL-negative cells (Table 5C). Thus, from our work, the HRP-labelling pattern cannot be explained by extensive cell recruitment from the ICM. The low incidence of cell division recorded in HRP-injected cells during the first 24 h period of culture noted both here (Table 5A) and demonstrated more convincingly with higher blastocyst numbers by Cruz & Pedersen (1985) suggests an alternative explanation. The temporary impairment in division capacity by HRP-injected cells may lead to their displacement laterally within the TE

by the proliferative action of noninjected neighbouring TE cells and to the formation of a HRP-negative central zone. Indeed, this explanation is supported by the observation that undivided HRP-injected cells after 24 h culture were invariably changed in their position relative to the embryonic: abembryonic axis from that occupied at the time of injection (Table 5B).

The detection of FL-negative polar TE cells in a small minority of cultured embryos may indicate that the delay between lineage restriction and commitment within the ICM may have a role in regulating embryonic development in cases of trophectodermal injury. Our failure to promote the incidence of an ICM contribution to TE following lesioning of the central polar TE cell argues against this possibility. It would appear that, in these damaged embryos (as in normal development), neighbouring viable TE cells manage to envelop and enclose any ICM cells that might have become exposed by injury to overlying TE before their capacity to differentiate can be realized. Thus, an explanation for the low incidence of lineage crossing detected in our experiments cannot be forwarded at present. It remains to be seen whether a role for ICM totipotency exists in embryos with extensive rather than localized trophectodermal injury.

In conclusion, our experiments reveal that TE and ICM lineages in mouse blastocysts become entirely separate in most, but not quite all, embryos soon after the onset of fluid accumulation. Segregation of lineages coincides with the 6th cell cycle and appears to be regulated by cellular interactions rather than by the termination of cell pluripotency.

We wish to thank Dr M. H. Johnson and our research colleagues for their helpful discussions; Gin Flach, Brendan Doe, Tim Crane and Roger Liles for technical work. The study was supported by an MRC grant to Dr M. H. Johnson and Dr P. R. Braude and a CRC grant to Dr M. H. Johnson.

References

Balakier, H. & Pedersen, R. A. (1982). Allocation of cells to inner cell mass and trophectoderm lineages in preimplantation mouse embryos. *Devl Biol.* **90**, 352–362.

Barton, S. C., Adams, C. A., Norris, M. L. & Surani, M. A. H. (1985). Development of gynogenetic and parthenogenetic inner cell mass and trophectoderm tissues in reconstituted blastocysts in the mouse. *J. Embryol. exp. Morph.* **90**, 267–285.

Beddington, R. (1983). The origin of the foetal tissues during gastrulation in the rodent. In *Development in Mammals*, vol. 5 (ed. M. H. Johnson), pp. 1–32. Amsterdam: Elsevier Science Publishers.

Borland, R. M. (1977). Transport processes in the mammalian blastocyst. In *Development in Mammals*, vol. 1 (ed. M. H. Johnson), pp. 31–67. Amsterdam, New York: North-Holland.

Chisholm, J. C., Johnson, M. H., Warren, P. D., Fleming, T. P. & Pickering, S. J. (1985). Developmental variability within and between mouse expanding blastocysts and their ICMs. *J. Embryol. exp. Morph.* **86**, 311–336.

Copp, A. J. (1979). Interaction between inner cell mass and trophectoderm of the mouse blastocyst. II. Fate of the polar trophectoderm. *J. Embryol. exp. Morph.* **51**, 109–120.

Cruz, Y. P. & Pedersen, R. A. (1985). Cell fate in the polar trophectoderm of mouse blastocysts as studied by microinjection of cell lineage tracers. *Devl Biol.* **112**, 73–83.

Ducibella, T., Albertini, D. F., Anderson, E. & Biggers, J. D. (1975). The preimplantation mammalian embryo: characterization of intercellular junctions and their appearance during development. *Devl Biol.* **45**, 231–250.

Ducibella, T. & Anderson, E. (1975). Cell shape and membrane changes in the eight-cell mouse embryo: prerequisites for morphogenesis of the blastocyst. *Devl Biol.* **47**, 45–58.

Ducibella, T., Ukena, T., Karnovsky, M. & Anderson, E. (1977). Changes in cell surface and cortical cytoplasmic organization during early embryogenesis in the preimplantation mouse embryo. *J. Cell Biol.* **74**, 153–167.

Fleming, T. P. (1987). A quantitative analysis of cell allocation to trophectoderm and inner cell mass in the mouse blastocyst. *Devl Biol.* **119**, 520–531.

Fleming, T. P., Cannon, P. M. & Pickering, S. J. (1986). The cytoskeleton, endocytosis and cell polarity in the mouse preimplantation embryo. *Devl Biol.* **113**, 406–419.

Fleming, T. P. & George, M. A. (1987). Fluorescent latex microparticles: a non-invasive short-term cell lineage marker suitable for use in the mouse early embryo. *Wilhelm Roux Arch. devl Biol.* **196**, 1–11.

Fleming, T. P. & Goodall, H. (1986). Endocytic traffic in trophectoderm and polarised blastomeres of the mouse preimplantation embryo. *Anat. Rec.* **216**, 490–503.

Fleming, T. P. & Pickering, S. J. (1985). Maturation and polarization of the endocytotic system in outside blastomeres during mouse preimplantation development. *J. Embryol. exp. Morph.* **89**, 175–208.

Fleming, T. P., Warren, P. D., Chisholm, J. C. & Johnson, M. H. (1984). Trophectodermal processes regulate the expression of totipotency within the inner cell mass of the mouse expanding blastocyst. *J. Embryol. exp. Morph.* **84**, 63–90.

Fulton, B. P. & Whittingham, D. G. (1978). Activation of mammalian oocytes by intracellular injection of calcium. *Nature, Lond.* **273**, 149–151.

Gardner, R. L. (1978). The relationship between cell lineage and differentiation in the early mammalian embryo. In *Genetic Mosaics and Cell Differentiation* (ed.

W. J. Gehring), pp. 205–241. Heidelberg, New York: Springer-Verlag.

Gardner, R. L., Papaioannou, V. E. & Barton, S. C. (1973). Origin of the ectoplacental cone and secondary giant cells in mouse blastocysts reconstituted from isolated trophoblast and inner cell mass. *J. Embryol. exp. Morph.* **30**, 561–572.

Gearhart, J., Shaffer, R. M., Musser, J. M. & Oster-Granite, M. L. (1982). Cell lineage analyses of preimplantation mouse embryos after blastomere injections with horseradish peroxidase. *Pediat. Res.* **16**, 111A.

Goodall, H. (1986). Manipulation of gap junctional communication during compaction of the mouse early embryo. *J. Embryol. exp. Morph.* **91**, 283–296.

Goodall, H. & Johnson, M. H. (1984). The nature of intercellular coupling within the preimplantation mouse embryo. *J. Embryol. exp. Morph.* **79**, 53–76.

Handyside, A. H. (1978). Time of commitment of inside cells isolated from preimplantation mouse embryos. *J. Embryol. exp. Morph.* **45**, 37–53.

Handyside, A. H. (1980). Distribution of antibody- and lectin-binding sites on dissociated blastomeres from mouse morulae: evidence for polarization at compaction. *J. Embryol. exp. Morph.* **60**, 99–116.

Handyside, A. H. & Johnson, M. H. (1978). Temporal and spatial patterns of the synthesis of tissue-specific polypeptides in the preimplantation mouse embryo. *J. Embryol. exp. Morph.* **44**, 191–199.

Hogan, B. & Tilly, R. (1978). *In vitro* development of inner cell masses isolated immunosurgically from mouse blastocysts. II. Inner cell masses from 3·5 to 4·0 day p.c. blastocysts. *J. Embryol. exp. Morph.* **45**, 107–121.

Johnson, M. H. (1979). Molecular differentiation of inside cells and inner cell masses isolated from the preimplantation mouse embryo. *J. Embryol. exp. Morph.* **53**, 335–344.

Johnson, M. H. (1986). Manipulation of early mammalian development: what does it tell us about cell lineages? In *Developmental Biology*, vol. 4 (ed. R. B. L. Gwatkin), pp. 279–296. New York: Plenum Publishing Corporation.

Johnson, M. H., Handyside, A. H. & Braude, P. R. (1977). Control mechanisms in early mammalian development. In *Development in Mammals*, vol. 2 (ed. M. H. Johnson), pp. 67–97. Amsterdam: North-Holland.

Johnson, M. H. & Ziomek, C. A. (1981). The foundation of two distinct cell lineages within the mouse morula. *Cell* **24**, 71–80.

Johnson, M. H. & Ziomek, C. A. (1982). Cell subpopulations in the late morula and early blastocyst of the mouse. *Devl Biol.* **91**, 431–439.

Johnson, M. H. & Ziomek, C. A. (1983). Cell interactions influence the fate of mouse blastomeres undergoing the transition from the 16- to the 32-cell stage. *Devl Biol.* **95**, 211–218.

Kimber, S. J., Surani, M. A. H. & Barton, S. C. (1982). Interactions of blastomeres suggest changes in cell surface adhesiveness during the formation of inner cell mass and trophectoderm in the preimplantation mouse embryo. *J. Embryol. exp. Morph.* **70**, 133–152.

Magnuson, T., Demsey, A. & Stackpole, C. W. (1977). Characterization of intercellular junctions in the preimplantation mouse embryo by freeze-fracture and thin-section electron microscopy. *Devl Biol.* **61**, 252–261.

Maro, B., Johnson, M. H., Pickering, S. J. & Louvard, D. (1985). Changes in the distribution of membraneous organelles during mouse early development. *J. Embryol. exp. Morph.* **90**, 287–309.

Nadijcka, M. & Hillman, N. (1974). Ultrastructural studies of the mouse blastocyst substages. *J. Embryol. exp. Morph.* **32**, 675–695.

Nichols, J. & Gardner, R. L. (1984). Heterogeneous differentiation of external cells in individual isolated early mouse inner cell masses in culture. *J. Embryol. exp. Morph.* **80**, 225–240.

Nicholson, G. L., Yanagimachi, R. & Yanagimachi, H. (1975). Ultrastructural localization of lectin-binding sites on the zona pellucidae and plasma membranes of mammalian eggs. *J. Cell Biol.* **66**, 263–274.

Papaioannou, V. E. (1982). Lineage analysis of inner cell mass and trophectoderm using microsurgically reconstituted mouse blastocysts. *J. Embryol. exp. Morph.* **68**, 199–209.

Pedersen, R. A., Wu, K. & Bałakier, H. (1986). Origin of the inner cell mass in mouse embryos: cell lineage analysis by microinjection. *Devl Biol.* **117**, 581–595.

Purves, R. D. (1981). *Microelectrode Methods for Intracellular Recording and Ionophoresis*, pp. 80–81. London: Academic Press.

Reeve, W. J. D. (1981). Cytoplasmic polarity develops at compaction in rat and mouse embryos. *J. Embryol. exp. Morph.* **62**, 351–367.

Reeve, W. J. D. & Ziomek, C. A. (1981). Distribution of microvilli on dissociated blastomeres from mouse embryos: evidence for surface polarization at compaction. *J. Embryol. exp. Morph.* **62**, 339–350.

Rossant, J. (1984). Somatic cell lineages in mammalian chimeras. In *Chimeras in Developmental Biology* (ed. N. Le Douarin & A. McLaren), pp. 89–109. London: Academic Press.

Rossant, J. & Croy, B. A. (1985). Genetic identification of tissue of origin of cellular populations within the mouse placenta. *J. Embryol. exp. Morph.* **86**, 177–189.

Rossant, J. & Lis, W. J. (1979). Potential of isolated mouse inner cell masses to form trophectoderm derivatives *in vivo*. *Devl Biol.* **70**, 255–261.

Rossant, J. & Vijh, K. M. (1980). Ability of outside cells from preimplantation mouse embryos to form inner cell mass derivatives. *Devl Biol.* **76**, 475–482.

Rossant, J., Vijh, K. M., Siracusa, L. D. & Chapman, V. M. (1983). Identification of embryonic cell lineages in histological sections of *M. musculus* ↔ *M. caroli* chimaeras. *J. Embryol. exp. Morph.* **73**, 179–191.

Spindle, A. I. (1978). Trophoblast regeneration by inner cell masses isolated from cultured mouse embryos. *J. exp. Zool.* **203**, 483–489.

Stern, M. S. & Wilson, I. B. (1972). Experimental studies on the organization of the preimplantation mouse embryo. I. Fusion of asynchronously cleaving mouse eggs. *J. Embryol. exp. Morph.* **28**, 247–254.

Surani, M. A. H. & Barton, S. C. (1984). Spatial distribution of blastomeres is dependent on cell division order and interactions in mouse morulae. *Devl Biol.* **102**, 335–343.

Surani, M. A. H. & Handyside, A. H. (1983). Reassortment of cells according to position in mouse morulae. *J. exp. Zool.* **225**, 505–511.

Whittingham, D. G. (1971). Culture of mouse ova. *J. Reprod. Fert. Suppl.* **14**, 7–21.

Ziomek, C. A. & Johnson, M. H. (1980). Cell surface interactions induce polarization of mouse 8-cell blastomeres at compaction. *Cell* **21**, 935–942.

Ziomek, C. A. & Johnson, M. H. (1981). Properties of polar and apolar cells from the 16-cell mouse morula. *W. Roux Arch. devl Biol.* **190**, 287–296.

Ziomek, C. A. & Johnson, M. H. (1982). The roles of phenotype and position in guiding the fate of 16-cell mouse blastomeres. *Devl Biol.* **91**, 440–447.

Ziomek, C. A., Johnson, M. H. & Handyside, A. H. (1982). The developmental potential of mouse 16-cell blastomeres. *J. exp. Zool.* **221**, 345–355.

(*Accepted 25 March 1987*)