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Original Article

Individual migration of mesentodermal cells in the early embryo of the squid *Loligo vulgaris: in vivo* recordings combined with observations with TEM and SEM

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ABSTRACT. In the translucent preorganogenetic embryo of the squid *Loligo vulgaris* a population of single cells between the ectodermal layer and the yolk syncytium can be studied continuously *in vivo* during migration to the vegetal hemisphere of the egg. The results from 2 different preparations are reported: 1. An intact embryo served to view locomotive cell behavior through the translucent ectoderm with undisturbed cell-substrate interactions. 2. In an embryo a patch of ectoderm was microsurgically removed thereby exposing migrating cells to direct observation and experimental manipulation. *In vivo* time lapse microcinematographic recordings for 22 h (in 1.) and 10 h (in 2.) revealed the following: cell migration is neither directional nor dependent on the presence of the ectodermal layer (in 2.). Although the migrating cells primarily use the syncytial surface as a substrate for locomotion, under natural conditions they also adhere to the basal ectodermal surface as revealed by TEM and SEM. Migration rates were 18.3±12.6 µ/h in 1. Locally directed cell migration was observed in a group of cells in 1. which were involved in a process of aggregration, the latter being probably related to precocious formation of organ primordia. A preliminary note has appeared previously (Segmüller and Marthy, 1984).

KEY WORDS: *cell migration* in vivo, *cell-substrate interactions, cephalopods, time-lapse microcinematography, TEM, SEM*

Introduction

The control and mechanism of the directed migration of single cells are fundamental concepts for the understanding of embryonic development. A general difficulty in investigating cell migration is the impossibility of observing living cells *in situ*, as in the migration of neural crest cells (reviewed in LeDouarin 1982; Weston 1983) or primordial germ cells (eg Meyer, 1964; Heasman *et al.*, 1985). Rare exceptions include primary mesenchyme cells in the translucent sea urchin embryo (Gustavson and Wolpert, 1967), Tunicate tunic cells (for references see Trinkaus, 1984) and melanophore migration in the axolotl embryo (Keller and Spieth, 1984). Extensive work has been presented by Trinkaus and his co-workers on a similar system namely the pregastrulation *Fundulus* fish embryo (Trinkaus, 1984). Although *in vitro* systems offer a variety of excellent possibilities for direct observation and experimental manipulation of migratory cells, cell-substrate interactions in the natural environment and the precise orientation of migration *in vivo* remain difficult to assess. In the preorganogenetic Cephalopod embryo active migration of single cells can be directly observed *in vivo* under both natural and experimental conditions. The examined individual cells are located in the future hemal space of the external yolk sac. They are derived originally from the edge of the mesentodermal complex (Marthy, 1982) and will ultimately differentiate into muscle cells of the external yolk sac, thereby persisting essentially as individual but linked cells rather than forming a solid muscular tissue (the rhythmic contraction of these cells causes the pulsation of the outer yolk sac as seen in organogenetic embryos). Here we report the results of *in vivo* recording assays from 2 embryos together with observation from Transmission and Scanning Electron Microscopy (TEM and SEM). Recordings on 3 other preorganogenetic embryos confirm the observations made, thus permitting the conclusion that the cellular behavior as described here is in fact true for preorganogenic squid embryos.

Results

Microcinematography

The results obtained from the intact embryo with the mesentodermal cells migrating in their natural environment between the ectoderm and the yolk syncytium, are compared with those from the operated embryo lacking a patch of ectoderm. In the operated embryo the recording period is mainly limited by the process of wound closure, which however does not start before the onset of organogenesis (stage IV to VII of Naef, 1928) i.e. recording is possible for up to several days depending on the culture temperature. The assay reported here covered a period of

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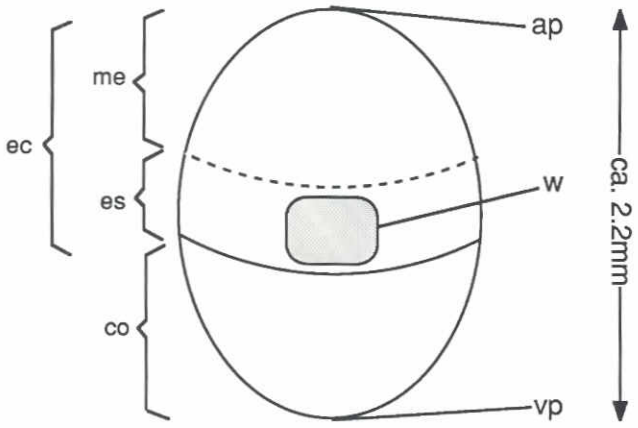


Fig. 1. *Morphological situation in the observed preorganogenetic embryo of* Loligo vulgaris *(stage V to VI, Naef, 1928). The large highly translucent yolk mass of the egg is partially (i.e. between about 1/2 and 2/3) overgrown by the progressing blastoderm which itself consists of an ectodermal layer (ec) covering the mesentodermal complex (me). Mesentoderm and ectoderm together form the embryonic part of the egg. The egg surface area covered only by the ectoderm constitutes an extraembryonic space (es) which will form the future external yolk sac envelope. Towards the vegetative (=vitelline) pole (vp) of the egg the original egg cortex (co) is not yet overgrown. ap: animal (=formative) pole. Excision of a patch of ectoderm in the extraembryonic space (w: window) reveals the yolk syncytium, which in this region is derived from the egg cortex, and single mesentodermal cells on top of it which have detached from the mesentodermal edge. These single cells, exposed in the ectodermal window (or visible through translucent ectoderm), and their locomotive behavior* in situ *are the subject of this study.*

10 h at stage V to VI (egg surface 1/2 to 2/3 overgrown by the blastoderm). In the intact embryo (same stage) the observation period was 22 h. The clearly visible syncytial nuclei continued to progress all through the recording period thereby indicating a normal course of blastoderm expansion.

With the objective used (x6) cellular details such as small pseudopodia or filopodia are not discernible. The overlying ectoderm in the intact embryo does not reduce significantly the visibility of the migrating cells except towards the periphery of the recording field, where both mesentodermal and ectodermal cells are out of focus. 25 cells in the operated embryo and 68 cells in the intact embryo were carefully followed in time lapse microcinematographic recordings (Fig. 2). Not all of the analyzed cells were traced throughout the respective observation periods. The migrating cells measure 9 to 22 µ in diameter in the living state. They appear slightly flattened; generally elongated (fibroblast type) or clearly rounded cell shapes(mitotic stages?) are rare. Migration rates, based on hourly interval tracing, were 18.3±12.6 µ /h in the intact embryo, with maximum values of up to 35 µ/h. In contrast, in the operated embryo, where a shifting movement of the syncytium due to a small lesion prevented systematic measuring, maximum migration rates were only 16 µ/h.

The mitotic rate of the migrating cells was 0.6% per hour (i.e. 9 of 68 cells divided within 22 h) in the intact embryo and 4.0% per hour (i.e. 10 of 25 cells divided within 10 h) in the operated embryo. In the latter case all mitotic divisions occurred in the second half of the recording period. Migrat-

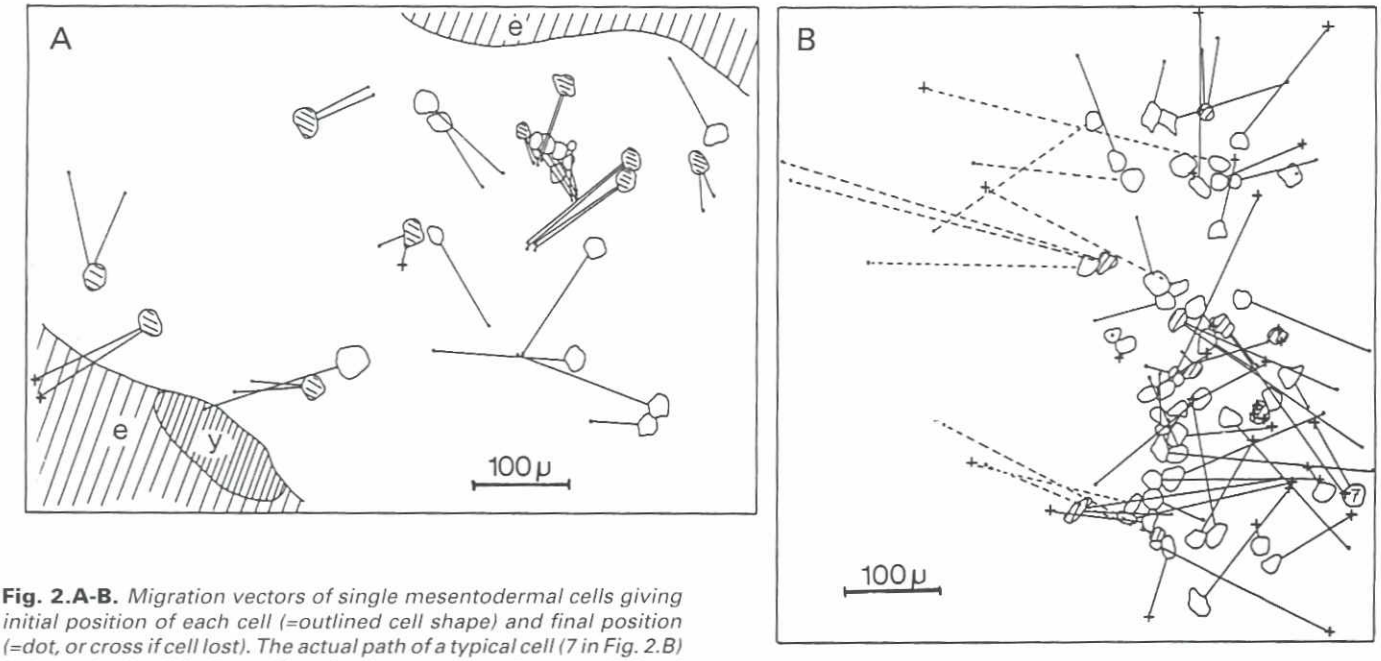


Fig. 2.A-B. *Migration vectors of single mesentodermal cells giving initial position of each cell (=outlined cell shape) and final position (=dot, or cross if cell lost). The actual path of a typical cell (7 in Fig. 2.B) is drawn in detail in Fig. 3. Situation in the operated embryo* ***(A)*** *and in the intact embryo* ***(B)****. Animal pole is towards top, vegetal pole bottom. Cells undergoing mitosis are drawn hatched. Migratory paths converging in an aggregation zone (in* ***B****) appear as broken lines. Ectodermal border e, accidental lesion in the yolk syncytium y (in* ***A****)*

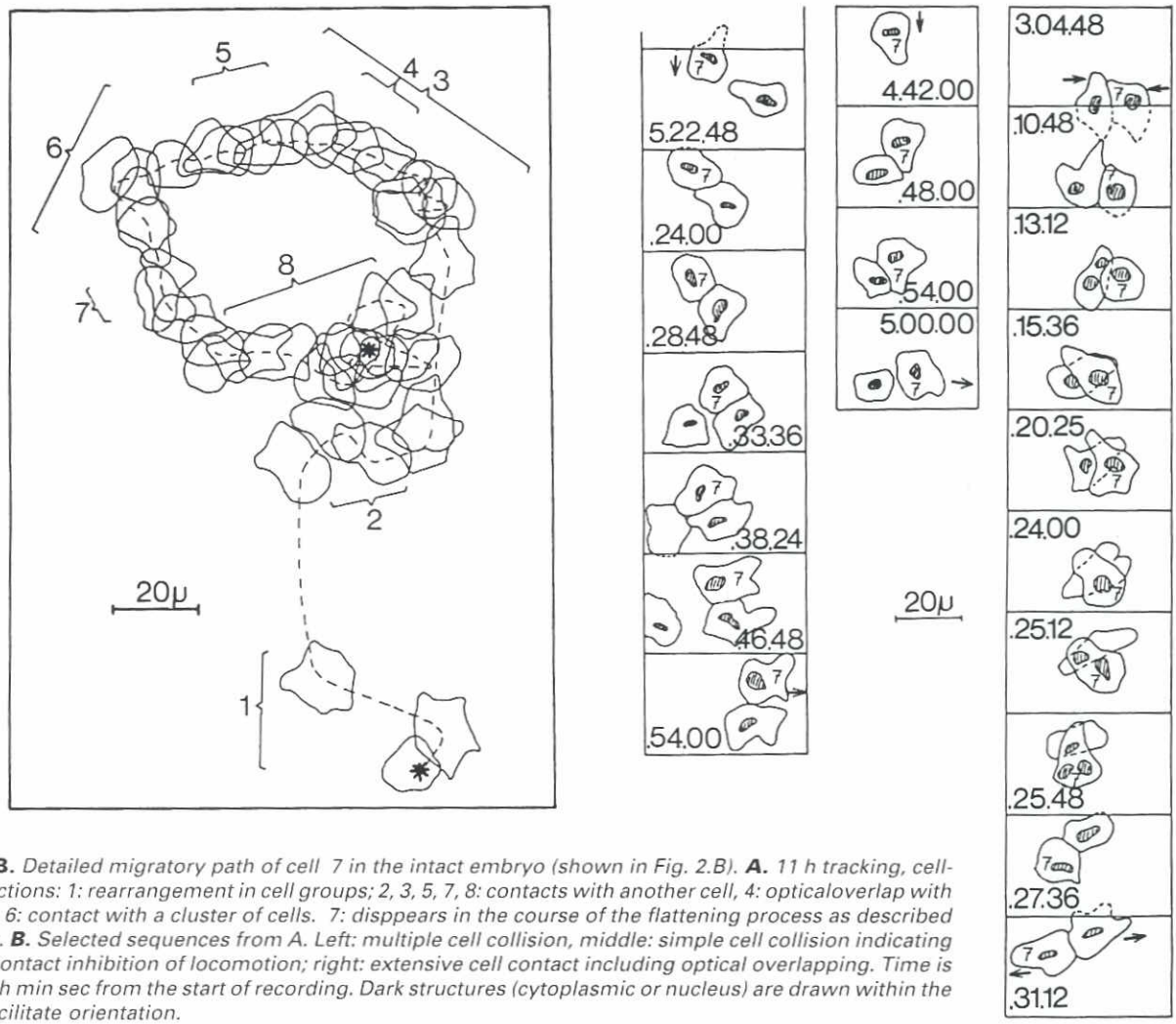


Fig. 3.A-B. *Detailed migratory path of cell 7 in the intact embryo (shown in Fig. 2.B).* ***A.*** *11 h tracking, cell-cell interactions: 1: rearrangement in cell groups; 2, 3, 5, 7, 8: contacts with another cell, 4: opticaloverlap with other cell, 6: contact with a cluster of cells. 7: disppears in the course of the flattening process as described in the text.* ***B.*** *Selected sequences from A. Left: multiple cell collision, middle: simple cell collision indicating possible contact inhibition of locomotion; right: extensive cell contact including optical overlapping. Time is shown as h min sec from the start of recording. Dark structures (cytoplasmic or nucleus) are drawn within the cells to facilitate orientation.*

ing cells stop locomotion at least 30 min before the onset of mitosis although violent membrane oscillations persist. Immediately after mitosis the daughter cells continue migration.

In the operated embryo there is no predominant direction for cell migration. However in the intact embryo there is a region in the animal hemisphere towards which a percentage of the migrating cells accumulate. Migration of cells in other directions is erratic (Fig. 2B). Migration efficiencies (calculated as the ratio between the migration vector i.e. the shortest start-to-end distance, and the total migration path length of a cell) are lower towards the vegetal than towards the animal hemisphere (Table 1). An alignment of cell migration to the animal-vegetal axis (Nakatsuji and Johnson, 1983) could not be demonstrated.

Although in general the cells migrate individually, not in groups, both mitotic divisions and accidental encounters between individual cells lead to the transient formation of small clusters of 2 to 6 cells during migration. These cell groups show strongly reduced mitotic and migration rates.

The role of contact inhibition of cell locomotion is examined in Fig. 3A by detailed tracing of cell 7 in the intact embryo throughout the 11 h it was visible. The cell collides with several other cells in the course of its migration. In collision-free intervals (between 25 min and 2 h each) the migration path is quite linear. The cell tends to maintain its original migration direction even during contact with another cell by migrating laterally past the other (Fig. 3B). In one such cell-cell collision a distinct optical overlapping of the 2 cells involved was observed (Fig. 3B). The same cell shows a complete reversion of its migration direction after a collision with a cluster of cells.

Some 30% of the cells (mostly in the intact embryo) "disappear" during observation. The process starts by a

TABLE 1

MIGRATION EFFICIENCIES IN THE INTACT EMBRYO

a. absolute migration efficiency

efficiency classes \ direction quadrants	↗	↖	↙	↘	total per class
0-.25	1	0	3.5	6.5	11
.26-.50	4.5	1	2.5	9	17
.51-.75	7	2	3	2.5	14.5
.76-1.0	4	1.5	1.5	1.5	8.5
total per quadrant	16.5	4.5	10.5	19.5	51

b. weighted relative migration efficiency

efficiency classes \ direction quadrants	↗	↖	↙	↘
0-.25	0.3	0	1.5	1.5
.26-.50	0.8	0.7	0.7	1.4
.51-.75	1.5	1.6	1.0	0.5
.76-1.0	1.5	2.0	0.9	0.5

1.a.The absolute migration efficiency is calculated as the ratio between the shortest distance from start to end point and the total path length of each cell (as measured by hourly interval tracing). The cells are grouped according to their absolute migration efficiency (4 classes) and to the direction of their migration (4 quadrants represented by arrows). After mitosis, daughter cells are counted as half.
1.b. The weighted relative migration efficiency is calculated as:

$$R = \frac{n}{\text{total per class}} \cdot \frac{51}{\text{total per quadrant}}$$

whereby n is the number of cells per class and quadrant (absolute efficiency), and 51 is the total number of all analysed cells.
In the first and second quadrant (i.e. upward migration= animal direction) highest efficiency values are located in the higher relative efficiency classes (.51-.75 and .76-1.0) whereas in the third and fourth quadrant the maxima are found in the low classes. Hence migration efficiency appears to be higher towards the animal than towards the vegetal hemisphere. Statistical significance of this difference has not been assessed.

gradual loss of visible activity of the cell followed by a slow and more or less distinct increase in cell diameter (up to twofold) and loss of optical contrast. Finally, after 15 to 30 min such a cell becomes indistinguishable from the substrate background. Other cells may soon thereafter migrate over the place previously occupied by the lost cell. In several other operated embryos (data not shown) up to 50% of the cells seemed to disappear suddenly within the 1 min interval between 2 single recording pictures.

Transmission Electron Microscopy

In TEM sections of intact stage V to VI embryos (1/2 of the egg surface overgrown by the blastoderm), isolated mesentodermal cells (Fig. 4) were identified as the migrating cells, observed in microcinematography. The morphological situation is very similar to the one described in the early *Fundulus* embryo by Betchaku and Trinkaus (1978: p. 385, stages 14-16). These cells have a ribosome-rich cytoplasm, few Golgi complexes and irregular shaped nuclei. The cell body in general is often rounded (eg 18*9 µ) although flattened forms occur (20*3.5µ). Small pseudopodia (1.3 µ long, Fig. 4F) indicate that the cells can adhere to either the basal ectoderm roof or the yolk syncytium, or both. Occasional junctional contacts are found between these mesentodermal cells and the syncytial yolk surface (Fig. 4H). The latter exhibits few microvillar protusions in this region, even underneath mesentodermal cells (Fig. 4G). Similar and other junctional complexes have also been described by Arnold and Williams-Arnold (1976).

The cells of the ectodermal layer show microvillar activity and cilia on the apical surface. Various kinds of junctional complexes (Fig. 4D; Ginzberg *et al.*, 1985) and cytoplasmic bridges (not shown; see Arnold, 1974; Cart-wright and Arnold, 1980; 1981) frequently occur. At the basal surface a basement membrane is poorly visible (Fig. 4E).

Scanning Electron Microscopy

After isolation of a patch of the ectoderm from the critical point dried embryo the exposed mesentodermal cells can be observed in SEM in the migrating state on both the syncytial yolk (Fig. 5) and the basal ectoderm (Fig. 6) surfaces. With the previously used technique an ectodermal patch was microsurgically excised from the living embryo prior to fixation (Marthy, 1982; for extensive SEM illustration see Marthy, 1985). However this simple double adhesive tape method, possibly also applied earlier on squid embryos (Arnold and Williams-Arnold, 1976; Fig. 9) considerably avoids washout of extracellular materials and the risk of precipitation artifacts during the fixation and dehydration procedures. Moreover this method permits a direct comparison of the 2 corresponding migration substrates: the large majority of the mesentodermal cells are found on the yolk substrate whereas only a few of them can be seen on the basal ectoderm surface (Figs. 5A and 6A). Accordingly the cells, as depicted in Figs. 5D and 6D,

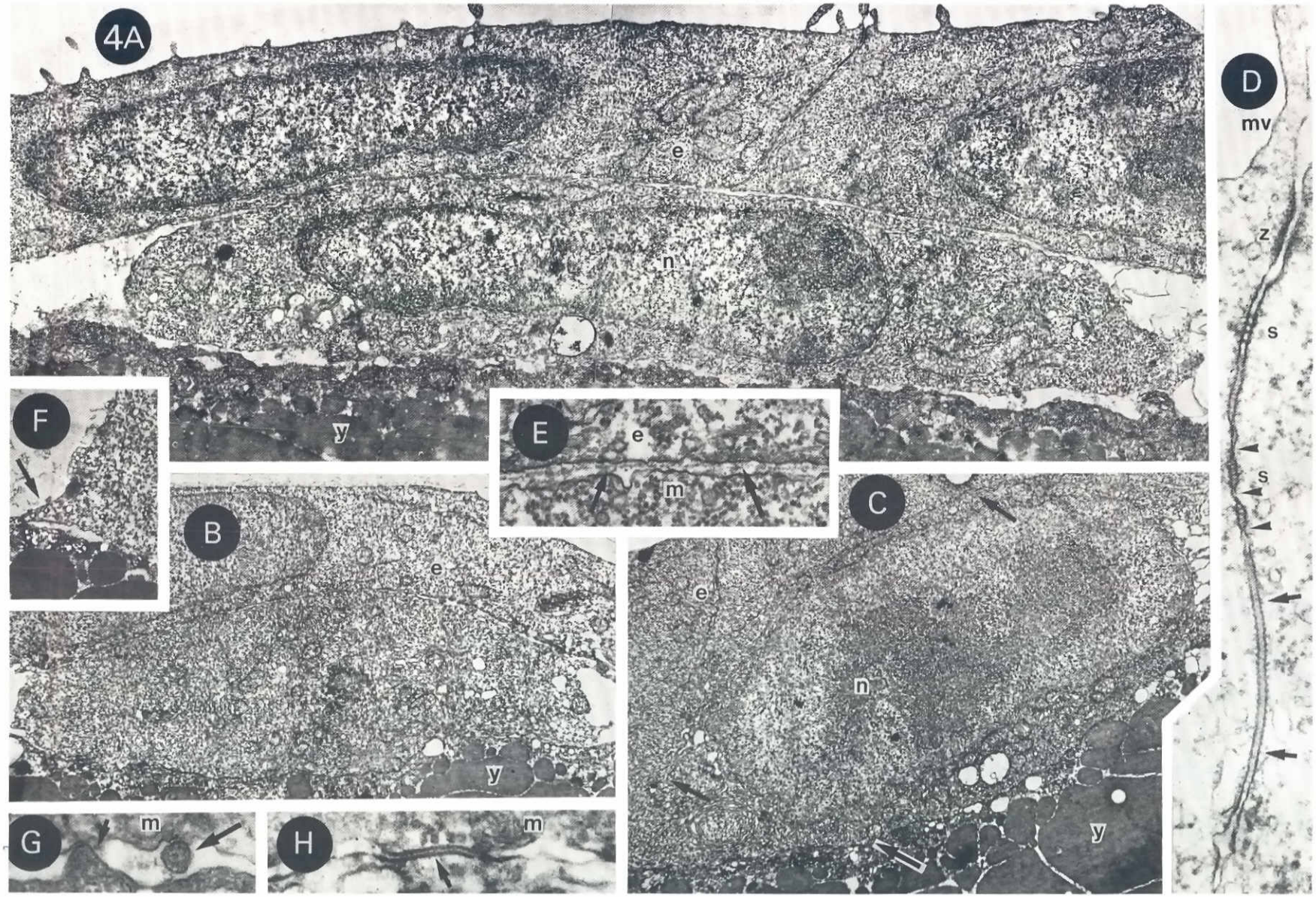
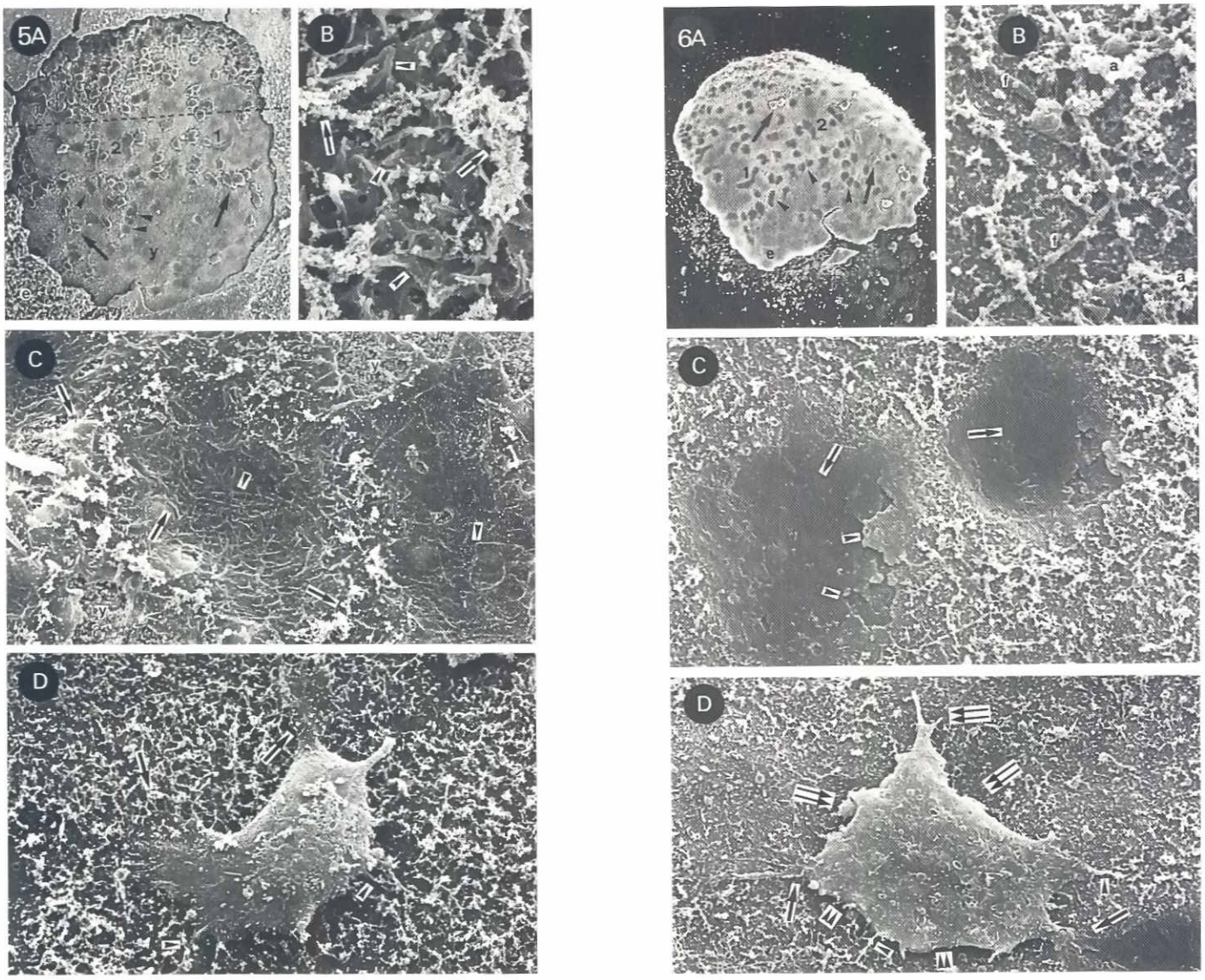


Fig. 4.A-H. *TEM micrographs of migrating mesentodermal cells in the extraembryonic space of an intact embryo, stage V (Naef, 1928), blastoderm 1/3 to 2/3 overgrown.* ***A-C.*** *Migrating cells covered by ectodermal layer (e) and lying on top of syncytial yolk surface (yolk granules: y).* ***A.*** *Flat cell body with prominent nucleus (n). x9500.* ***B.*** *Cytoplasmic section. x5600.* ***C.*** *Rounded cell body with large nucleus (n). Arrows indicate cell border. x5600.* ***D.*** *Elaborate junctional complex in the ectodermal layer. Microvillar protrusion (mv), zonula adherens (z), conventional septate junction s, septate junction of unknown type (arrows), close membrane contacts probably represent tight junctions (arrowheads). x53000.* ***E.*** *Ectodermal substrate for mesentodermal cell migration. Extracellular matrix forming a basal lamina (arrows) at the basal ectoderm surface (e). Mesentodermal cell (m). x39000.* ***F-H.*** *Syncytial substrate.* ***F.*** *Pseudopod-like cytoplasmic protrusion (arrow) of a mesentodermal cell attached to the yolk syncytium. Similar pseudopods are found attaching to the basal ectoderm surface (not shown). x7700.* ***G.*** *Microvillar protrusions of the syncytial yolk surface (arrows) underneath mesentodermal cell (m). x58200.* ***H.*** *Junctional contact (arrow) between yolk syncytium and mesentodermal cell (m). x61200.*



Figs. 5 and 6. *Comparison of migrating mesentodermal cells on the syncytial yolk (5) and the basal ectoderm (6) substrates. SEM microphotographs, stage V to VI, blastoderm 1/2 overgrown. Preparation as described in Materials and Methods, magnification rates corresponding in series 5 and 6.* ***5.A.*** *Mesentodermal cells (arrows 1-2) exposed on the yolk surface after removal of a patch of ectoderm (shown in 6.A) from the critical point dried embryo. Ectoderm (e); transition zone between embryonic and extraembryonic space (dotted line); syncytial yolk surface (y) (as shown in 5.B); places where mesentodermal cells adhering more strongly to the ectoderm have been torn off (arrowheads, as shown in 5.C). Orientation of the embryo: animal (i.e. formative) egg part is on top, vegetal (i.e.vitelline) egg part at the bottom as in Fig. 1. x160.* ***6.A.*** *Patch of ectoderm removed from embryo shown in 5.A with adhering mesentodermal cells (arrows) on its basal surface (e), shown in 6.B. Traces of cells adhering to the yolk surface (arrowheads, 1-2, shown in Fig. 6.C. x160.* ***5.B.*** *Syncytial yolk surface from 5.a consisting of numerous microvillar protrusions (mv, arrowheads). Amorphous material (arrows) covering the surface is thought to represent extracellular matrix components (ecm) filling up the interdermal space: from the preparation technique used here the formation of precipitation artifacts appears very unlikely. x10,000.* ***6.B****. Basal ectoderm surface from 6.A showing both fibrillar (f) and amorphous (a) ecm which probably is part of the thin basal lamina seen in 4.E. Note absence of mv. x10,000.* ***5.C*** *Small depressions in the syncytial yolk surface indicate the sites where mesentodermal cells have been torn off. Amorphous ecm, (arrows) is visible at the outlines of removed cells. Flat mv (arrowheads), free from ecm, coat the inside of the depressions. Where the syncytial layer has been torn off nude yolk granules (y) appear. x3,700.* ***6.C.*** *Analogous depressions in the basal ectoderm surface. Inside of depressions (arrows) is free from both the fibrillar and amorphous ecm as encountered outside them. Part of another layer (of ecm?) can be seen detaching (arrowheads) inside the depressions. x3,700.* ***5.D.*** *Typical migrating mesentodermal cell on the syncytial yolk substrate. Note threadlike filopodia (arrowheads) and large lobopodia (arrows) spreading into the mesh of mv and ecm particles. Close interaction of cellular protrusions with syncytial substrate structures indicates an active role of this substrate in mesentodermal cell locomotion (cf. Marthy, 1985: p. 172 etc.). x2,800.* ***6.D.*** *Similar migrating cell on the basal ectoderm substrate. Note filopodia (arrowheads) and small lobopodia (arrows) attaching to the ectodermal surface whereas most of the cell border is not in direct contact with the (ectodermal) substrate (double arrows). Apparently broken protrusions (double arrows, double arrowheads) indicate simultaneous adhesion to the syncytial yolk substrate. x2,800.*

show signs of a stronger adhesion to the syncytial than to the ectodermal substrate. The latter lacks the extensive microvillar protrusions found on the yolk surface.

An interesting feature are the "holes" left by the mesentodermal cells on the opposite substrate i.e. for each cell adhering to the yolk syncytium a small depression exists on the corresponding ectoderm surface where the cell has been torn off the ectoderm and vice versa (Marthy, 1985: p. 180). In such depressions (Figs. 5C, 6C) the respective substrates are devoid of those extracellular materials which otherwise surround the mesentodermal cells. Instead "flat" microvilli become visible on the yolk surface, and a presumably extracellular layer lacking prominent morphological structures appears on the basal ectodermal surface. These seem to be the very substrates to which the cells adhere *in vivo*. The cells are quite evenly distributed on the syncytial surface. Many of them exhibit large lobopodia and numerous filopodia (Figs. 5D, 6D). They resemble epithelial cells migrating on glass or plastic substrates. Most of them are spread on their substrate. Pairs and small groups of cells are connected by cytoplasmic bridges (not shown in detail, see Arnold, 1974; Cartwright and Arnold, 1980 and 1981; Marthy, 1982 and 1985). A second fraction of cells, showing a distinct rounded shape and threadlike retraction fibers, is thought to represent various mitotic stages (not shown in detail; see Marthy, 1985: p. 184).

No predominant orientation of cell polarization is evident in SEM. This confirms the situation found in the microcinematographic preparations.

Discussion

The Cephalopod embryo offers two possibilities to study single cell migration *in situ* and these are shown in this paper: firstly, in the intact embryo the migrating mesentodermal cells can be observed through the translucent ectodermal layer covering them. This permits the observation of cells under natural conditions, but experimental manipulations are very much restricted. Trinkaus and his co-workers have been studying an analogous system in the early *Fundulus* embryo (Trinkaus, 1978). Secondly, direct access to the migrating cells is possible in living Cephalopod embryos where a patch of the overlying ectoderm is removed. This permits experimental manipulation directly on the cells but the question arises how far the migrating cells under these modified conditions reflect a "natural" behavior.

In our assays with both intact and operated preorganogenetic Cephalopod embryos we demonstrate that continuous *in vivo* observation is feasible for at least 22 h (intact) or 10 h (operated) respectively. Similar, prolonged, *in vivo* observations of migrating embryonic cells are only reported rarely, for example, in the Killifish (immigration of mesenchymal cells into the developing pectoral fin, (Wood and Thorogood, 1984) or for pigment cells in the axolotl (Keller and Spieth, 1984).

Earlier excision experiments (cf. Marthy, 1978) have shown that no developmental consequences are to be expected from local removal of ectoderm in these preorganogenetic stages (stage V to VI of Naef, 1928).

The orientation pattern of the migrating cells is ambiguous: in both the intact and the operated embryo a majority of the mesentodermal cells tend to become evenly distributed on the vegetal hemisphere of the egg (colonizing population), but in the intact embryo a smaller group of these cells is involved in an aggregation process which can best be interpreted as an early stage of organ primordia formation (eg. arm bud in the equatorial egg region). In both cases cell migration is active (Nieuwkoop and Sutasurya, 1979: p. 113) i.e. the locomotive cells are moving relative to their environment. As to the colonizing population, although it statistically moves from its starting point in the animally located mesentodermal complex down to the vegetal hemisphere, no directionality is found in the recorded migration paths of the involved single cells in both preparations. Instead, erratic migration paths and consequently low migration efficiencies indicate an apparent tendency of the cells to minimize contact with each other. This results in a diffusion-like mechanism of migration. A possible basis for such a cell behavior can be seen in the (chemotactic) repulsion model as proposed by Twitty and Niu (1954). As to the aggregating cell population in the intact embryo assay, the cells involved show a clearly directed locomotive behavior as is also reflected by the increased migration efficiency in the direction of the aggregation movement. As a mechanism underlying this aggregation process one could imagine some kind of chemotactic attraction. There is no evidence for contact guidance of cell migration, neither in the colonization nor in the aggregation process.

What is the particular role of the yolk syncytium and the basal ectoderm surface for Cephalopod mesentodermal cell locomotion? Undoubtedly the yolk syncytium acts as the primary substrate for the migrating cells. Continued cell movement after operating the embryo, as well as the fact that most cells in our SEM preparations sit on the yolk substrate rather than on the ectoderm, support this view. Nevertheless the observed changes in mitotic rate and migratory activity in the operated embryo together with our TEM and SEM data imply a role of co-substrate for the ectoderm. The modification of the environment resulting from removal of an ectoderm patch, may itself cause some kind of unspecific activation of the mesentodermal cells which then leads to the observed behavioral changes.

Migrating cells are frequently observed to "disappear" during recording. They may represent cells which undergo flattening in the course of their final differentiation into single but finally interconnected muscle cells. If this is the case differentiation of the migrating cells should not be a synchronous event at the end of migration but rather a continuous process throughout the migration period. On the other hand, instead of starting differentiation, such

cells may also be in the process of necrosis as their normal developmental fate. There may be other possibilities (embryonic blood cells?). Neither in TEM nor in SEM preparations were we able to find a hint as to what really happens to these cells. Further studies focusing on this particular problem are currently under way.

Materials and Methods

Biological model

Egg strings of *Loligo vulgaris* were collected in the Mediterranean Sea near Banyuls-sur-Mer, France, using trawling nets at depths of 50 to 100 m. They were maintained in running sea water (15±2°C) until they reached stage V to VI of Naef (1928) in which the blastoderm covers about 1/2 of the surface of the yolk syncytium (Fig. 1). The surrounding egg jelly and chorion were removed from suitable embryos in filtered sea water with watchmaker's forceps. A thin layer of agar avoided sticking of the nude embryos to the bottom of the Petri dish. If cultured in sterile sea water (with 50-100 U/ml Penicillin) such embryos develop normally to hatching stage (Marthy, 1970).

Experimental techniques

For *in vivo* time-lapse microcinematography the migrating mesentodermal cells were observed through the translucent ectodermal layer in an intact embryo. Other embryos were subjected to microsurgery before recording by slightly modifying the technique of Marthy (1978, 1982), i.e., a small patch of ectoderm was excised with tungsten needles to expose the underlying mesentodermal cells (Fig. 1). During this operation and subsequent recording the position of the embryo was maintained in a small hole in the agar bottom. For recording, a Leitz Ortholux microscope (bright-field optics, objective x6) was used in combination with a Bolex HRX16 camera and time lapse equipment (Paillard-Wild, Heerbrugg, Switzerland) at 20±1°C and a rate of 1 frame/min. In the squid embryo as a whole mount preparation, optical quality of recording was limited by opacity of the large central yolk mass underneath the observed cells which also prevented the use of phase contrast optics.

For TEM, intact embryos were fixed with glutaraldehyde and osmium tetroxide according to the method of Eisenman and Alfert (1981) for marine invertebrate tissues, dehydrated through a graded series of ethanol and propylene-oxide, and embedded in a hard Epon mixture (Millard de Montrion, 1984).

For SEM, intact specimens were fixed with 1% OsO4 in sea water for 2 to 3 h, dehydrated with ethanol, and critical-point dried with CO_2. By gently applying double-sided adhesive tape to the dried embryos a small patch of ectoderm was torn off with the tape thereby exposing the syncytial surface on the embryo as well as the corresponding basal surface of the ectoderm patch. Migrat-ing mesentodermal cells were found on both these surfaces. Embryos so prepared were sputtered with 20 nm gold.

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