SUMMARY

Circus movements, which involve the circumferential rotation of a hyaline cytoplasmic protrusion, occur in cells obtained by EDTA dissociation of gastrula-stage *Xenopus laevis* embryos. Only a few dissociated blastula-stage cells show circus movements, more early gastrula-stage cells show them, and nearly all late gastrula-stage cells show them. Circus movements cease in cells prior to mitosis and begin again in daughter cells after mitosis is completed. In early gastrulae, only 17% of prospective endodermal cells show circus movements while 79% of prospective mesoderm, archenteric roof, and posterior neural ectoderm do so. Isolated cells as well as groups of cells in vitro are often propelled by circus movements. There is an obvious antagonism between cell contact and circus movements. The morphogenetic significance of circus movements and blebbing locomotion is discussed.

INTRODUCTION

Dissociated embryonic cells show different behaviour *in vitro* depending upon a number of variables including developmental stage of embryos, dissociation method, culture conditions, and region in the embryo of cell origin. Holtfreter (1946, 1947) presented a detailed descriptive analysis of the behaviour of dissociated embryonic amphibian cells. One of the most interesting motile phenomena shown by dissociated embryonic cells is the so-called circus movement which involves a circumferential rotation of a protruding hyaline bleb. Circus movements in amphibian embryonic cells have been described (Roux, 1894; Holtfreter, 1946; Karasaki, 1957; Sirakami, 1959, 1963; Kauffman, 1974) qualitatively and quantitatively, but little is known about the morphogenetic significance, if any, of this motile behaviour. The present study confirms previous descriptive accounts, and presents new data on circus movements in cells from different developmental stages and different parts of *Xenopus* gastrulae. In addition, new observations on lobopodial cell movements in undissociated and reaggregating multicellular fragments suggest a morphogenetic role for circus movements and other kinds of blebbing behaviour.
MATERIALS AND METHODS

*Xenopus laevis* were obtained from Charles W. Fletcher (Glen Burnie, Md) and induced to breed as described by Gurdon (1967). Embryos were collected at the 2-cell stage and then the time of fertilization was determined, knowing the length of time required to reach the 2-cell stage. Embryos were staged according to Nieuwkoop & Faber (1967) and raised in 10% modified Steinberg's solution (formula in Johnson, 1970) at 21–23 °C. Jelly coats were removed by a 5-10 min treatment in 0.7% mercaptoacetic acid in modified 50% Steinberg's solution with the pH adjusted to 8.6 with 5 N NaOH after addition of mercaptoacetic acid. After jelly coats were removed, embryos were raised at 23 °C in 10% Steinberg's solution. For dissociation, embryos were transferred to Ca²⁺, Mg²⁺-free Steinberg's solution (CMF), and fragments of embryos were isolated with sharpened no. 5 Dumont watchmaker's forceps. These fragments were incubated for 30 min in 1 mM EDTA in CMF and then expelled from a Pasteur pipette into a reservoir of Steinberg's solution with 0.5% bovine serum albumin (BSA) (Fraction V, Sigma) added. The reservoir was made on an acid-washed microscope slide with a rectangle of Vaseline to contain the medium. After 1 min, cells had settled out of suspension and the EDTA was washed away with 3 changes of fresh BSA. The slide was then sealed with a coverslip and examined under phase-contrast or Nomarski optics. Cells were photographed on 35-mm Ilford Pan-F film or 16 mm Kodak Plus X Reversal movie film at 16 frames/min. Histological methods have been described previously (Johnson, 1970). Mitotic index was determined as the ratio of nuclei in metaphase to total number of nuclei in randomly chosen portions of the roof of the blastocoel of mid-sagittal sections of embryos.

RESULTS

Circus movements

When a fragment of the roof of the blastocoel of a late gastrula (Stage 12) is dissociated and put in culture, hyaline lobopodia protrude from the cell surface and begin to move around the cell periphery. As a wave of hyaline cytoplasm passes around the cell, first small endoplasmic particles and then larger yolk platelets flow into the formerly hyaline lobopodium. This travelling bleb moves at 0.9 ± 0.2 (S.D.) μm/s in either clockwise or counter clockwise direction. Sometimes, hyaline blebs travel under a cell and move the entire cell away from its location. This indicates that the blebs can adhere to a glass substratum and exert a force large enough to move the cell. Fig. 1 shows 4 cells from a late gastrula-stage embryo photographed at 30-s intervals. All circus movements are qualitatively similar, regardless of stage or embryonic region of origin.

Circus movements and mitosis in different developmental stages

When a fragment of a gastrula-stage embryo is treated with 1 mM EDTA for 30 min, dissociation is rapid and nearly complete. As cells are released from the fragment during dissociation, circus movements begin. After dissociation, however, not all cells are engaged in circus movements. The percentage of the population involved in circus movements rapidly increases to a plateau value within 15 min after placing the cells in culture (Fig. 2). Determinations of the percentage of a population of cells exhibiting circus movements were made for prospective ectodermal and prospective neural plate cells taken from embryos of different developmental stages. Only 5–8% of the
Circus movements in frog gastrula cells

Fig. 1. Sequence of photomicrographs illustrating circus movements in 4 cells in a preparation made from a fragment of prospective ectodermal tissue from a late gastrula (Stage 12). In the first photograph, cells had been in culture for 15 min, and the subsequent ones are at 30-s intervals. The cell in the upper left corner has a rotating bleb moving counterclockwise for the first 3 frames and clockwise for the next three. The cell in the upper right has a bleb which rotates counterclockwise in all 6 frames. The cell in the lower left has a bleb which is not rotating in any of the 6 frames but begins to rotate in the last one. The cell in the lower right has a bleb rotating clockwise for the first 4 frames and then not moving in the last two. Scale bar = 50 μm.

cells taken from 6-, 7-, and 8-h blastulae (Stages 8 and 9) show circus movements when scored 30 min after dissociation. In contrast, 42% of the cells taken from 9 h early gastrulae (Stage 10) show circus movements and increasing percentages of the cells taken from 10-, 11-, 12-, and 13-h gastrulae (Stages 10⁺, 11⁺, 11 and 12) show circus movements. The mitotic index is relatively high during the blastula period.
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and decreases rapidly in the gastrula period (Fig. 3). Numerous instances have been observed where blastula- and gastrula-stage cells in culture will show circus movements for a time, become inactive and divide; then circus movements are shown again by the daughter cells. There is an obvious antagonism between mitosis and circus movements.

![Graph showing frequency of circus movements](image)

**Fig. 2.** Frequency of circus movements in a population of cells taken from 10 h (Stage 10+) embryos. At the beginning of the counting, cells were 10.5 h old, since dissociation was for 30 min. The first 100 cells encountered on a slide were scored in 10 preparations. The points are the averages of 10 determinations and the bars represent the standard deviations.

**Circus movements in different regions of early gastrulae**

Early (9-h, stage 10) gastrulae were dissected into 4 fragments composed predominantly of prospective endodermal cells (fragment I), prospective posterior and lateral ectodermal cells (fragment II), prospective head and neural ectoderm (fragment III), and prospective mesoderm, archenteric roof and posterior neural ectoderm (fragment IV) (Keller, 1975, 1976). The approximate locations of the 4 fragments are indicated in Fig. 4. Each fragment was dissociated in EDTA and cells were cultured as described above. Five preparations were scored for percentages of cells engaged in circus movements after 30 min *in vitro*. Only 19 ± 6% (s.d.) of the prospective endodermal cells (fragment I) taken from an early gastrula show circus movements. The rest are rounded up and show no surface activity. More cells (51 ± 7%) from fragment II show circus movement in the early gastrula when compared to fragment I. Similar results are found for fragment III cells (62 ± 6%). Most of the cells taken from fragment IV exhibit circus movements at the early gastrula stage (79 ± 7%).
Behaviour of groups of cells in vitro

The dissociation procedures used here occasionally result in some undissociated cell clusters in preparations. In such clusters, mobile hyaline blebs form and move on the cell periphery in places where there are gaps between cells but not in regions of cell contact. Intact sheets of cells show hyaline bleb formation only at free edges. Also, as isolated cells in culture reaggregate, hyaline bleb formation is suppressed at sites of contact. There appears to be antagonism between cell adhesion and hyaline bleb formation (Lucy & Curtis, 1959). This antagonism is best revealed by time-lapse studies of reaggregation in vitro. Soon after dissociation, groups of cells are irregular in shape and have points of contact in only a few places. They tend to be rounded up and at the same time form many hyaline blebs. The cells are in contact and also blebbing very actively where not in contact. The net result is active writhing of the entire mass of cells (Fig. 5). The edges of such masses of cells move at an average rate of about 1 μm/min over a 10-min period but sporadically move much faster. As cells reaggregate by increasing their area of cell contact, they become more polygonal.

Fig. 3. Frequency of circus movements (○) and mitotic index (●) in populations of cells from the roof of the blastocoel (prospective ectodermal and prospective neural plate) of different developmental stages. Each point was determined by taking an embryo 1 h younger than indicated on the graph, dissociating it for 30 min, leaving the cells in culture for 30 min, and then scoring quickly the first 200 cells encountered on a slide. Miotic indices were determined on embryos which were fixed at the hours indicated. Cells in mid-sagittal sections from 5 different embryos of the indicated ages were then scored by counting 500 nuclei in each embryo. The points on the graph are the averages of 5 determinations and the bars represent the standard deviations.
and show less hyaline bleb formation. The entire mass of cells becomes more compact and rounded, and less active. Moving blebs are apparently capable of forming transient adhesions with other cells and then moving these cells. Also, a cell can use a hyaline bleb to move by pushing away from another cell. It is clear from film analysis that hyaline bleb formation is an active process capable of exerting forces on the cells generating the bleb as well as on nearby cells. It is accurate, therefore, to consider these hyaline blebs to be cell extensions involved in cell locomotion.

DISCUSSION

Circus movements in dissociated embryonic amphibian cells begin at the time of onset of gastrulation, following a period of intense cell division. In gastrula-stage embryos, many cells taken from areas of the embryo directly involved in morphogenetic cell movements show active circus movements, while only a few cells taken from areas of the embryo not directly involved in morphogenetic cell movements show active circus movements. Also, it is clear that hyaline blebs adhere to both glass substrata and other cells and can generate enough force to move either the cell producing the bleb, or other groups of cells. Blebbing activity in groups of adhering cells in vitro can also change the gross shape of the entire mass within a few minutes. These results suggest that blebbing is an important driving force for morphogenetic cell movements in amphibian gastrulation. We do not know if this kind of activity occurs in vivo in Xenopus embryos. However, Nakatsuji's recent histological work
Fig. 5. Tracing from a time-lapse record of mass cell movement driven by lobopodial activity. The solid line is the outline of the mass of cells after 30 min of reaggregation in vitro. The dotted line is the outline of the same mass of cells 10 min later. The double arrows point to 3 locations where hyaline blebs are forming and moving the mass of cells indicated by the solid line. The single arrows point to 4 locations where hyaline blebs are forming and moving the mass of cells indicated by the dotted line. The stippled circle is the nucleus of a cell visible in the mass of cells indicated by the solid lines. The scale bar represents 20 μm.

(1974, 1975a, b) shows that involuting cells near the blastopore in gastrula-stage amphibian embryos have hyaline blebs of irregular shape which, in many instances, look as if they are the same as the rotating blebs formed by dissociated Xenopus cells in vitro. It seems likely that circus movements of blebs also occur in vivo in amphibian embryos, although no direct evidence is available at present. Nakatsuji (1975a) also showed that masses of cells move at rates of 1–6 μm/min during gastrulation in Xenopus. In the present studies, the edge of a moving mass of cells in vitro moves at an average rate of about 1 μm/min during 10-min intervals but sporadically moves at rates of about 5 μm/min for periods less than a minute. This suggests that the rates of mass cell movement, in vitro, generated largely by bleb formation, are high enough to account for rates of mass cell movement in vivo during gastrulation.

Direct observations of blebbing motility of cells in undisrupted frog embryos are impossible, since they are not transparent. Trinkaus (1973) has made use of transparent Fundulus embryos to show that deep cells show circus movements in vivo and that lobopodia are of significance in the locomotion of deep cells in vivo. Also, lobopodial activity and locomotion become more intense in deep cells prior to the beginning of epiboly. The locomotion of deep cells has morphogenetic significance as a way of remodelling a collection of cells during gastrulation. Circus movements in cells from...
dissociated blastoderms of *Oryzias latipes* have been reported to occur *in vitro* (Fujinami & Kageyama, 1975). These are very similar to the circus movements described for amphibian cells.

*In vivo*, embryonic amphibian cells are invariably in contact with other cells rather than being in complete isolation. From the *in vitro* observations it seems probable that when hyaline blebs form *in vivo*, they move on the cell periphery until they contact another cell. Upon contact, a hyaline bleb then probably either stops, moves the cell it contacts, moves the cell which generated the bleb, or moves a group of cells. However, exactly what happens after a hyaline bleb contacts another cell *in vivo* is unknown. Isolated cells *in vitro* probably form hyaline blebs which rotate around the cell because there is no other cell to contact the bleb and modify its behaviour. It appears that hyaline bleb formation might be one of the important driving forces for morphogenetic cell movements. If this is so, a major unanswered question of the control of morphogenesis is how this apparently disorganized pushing and pulling behaviour is coordinated into organized morphogenetic cell movements.

This study was begun in the Department of Biology, Yale University and was supported by an ND3A Title IV predoctoral fellowship and NSF Grant GB 4265X to Dr. J. P. Trinkaus. It was completed at Duke University and was supported by NIH Grant HD 07082 to Dr Kurt E. Johnson. Drs S. J. Counce, J. P. Trinkaus, and R. E. Keller provided helpful advice on the manuscript. The author wishes to express his gratitude for this support and help.

REFERENCES


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(Received 6 April 1976)