CIRCUS MOVEMENT IN DISSOCIATED EMBRYONIC CELLS OF A TELEOST, ORYZIAS LATIPES

NOBORU FUJINAMI AND TETSUO KAGEYAMA

Laboratory of Developmental Biology, Zoological Institute, Faculty of Science, University of Kyoto, Sakyo-ku, Kyoto 606, Japan

SUMMARY

The dissociated early embryonic cells of the fresh water fish, Oryzias latipes, protrude hyaline lobopodia, which tend to rotate around the cell circumference in a propagating wave. Cells from late blastula or gastrula continuously show this ‘circus movement’, while most cells up to early blastula are rounded. The linear velocity of the lobopodium was estimated by means of time-lapse cinemicrography. The velocity increases slightly as cell diameter increases.

The effects of pH, temperature and osmotic pressure of the immersion media on the movement were also quantitatively investigated. Cells become rounded and do not form lobopodial blebs when immersed in media below pH 5. The velocity is reduced by decreasing temperature, but the movement continues even at 5 °C. Cells placed in hypertonic salt solutions become crenated and do not continuously demonstrate the circus movement.

INTRODUCTION

The process of circus movement involves blebbing of a hyaline pseudopodium, spreading of the bleb, and thus propagating of the lobopodium around the cell circumference. This movement has been observed in various cells such as amoebae (Rhumbler, 1898), dissociated gastrodermal cells of planaria (Betchaku, 1967), eggs of a surf-clam (Rebhun, 1963), amoebocytes in the circulating blood of a crab (Loeb, 1928), embryonic teleostean cells in vitro (Trinkaus, 1973; Kageyama, 1975) and in vitro (Sirakami, 1963), dissociated embryonic amphibian cells (Roux, 1894; Holtfreter, 1946; Karasaki, 1957; Sirakami, 1959, 1963; Johnson, 1970; Kauffman, 1974), and chick fibroblasts in culture (Dornfeld & Owczarzak, 1958). Thus, the circus movement may be regarded as common behaviour of many diversified animal cells, based on the fundamental properties of their cell surfaces and motile activities. In addition to the cytological interest as a form of amoeboid movement, this behaviour of metazoan embryonic cells also attracts attention due to its embryological aspects.

This paper is dedicated to the memory of our Professor Ken-Iti Sirakami (1913-1974) who had been interested in the circus movement for many years. He measured the velocity of circus movement in various species of amphibians and attempted to associate those values with the progressive velocities of the surface cleavage furrow and the overall embryonic developmental sequence. His efforts are beneficial to the understanding of Dettlaff’s description (1964) and it will offer us a prospect of seeing embryological processes in terms of activities of individual cells sub specie moti.
such as its relationship to the morphogenetic movements of the embryo or to the
cell differentiation. The process of circus movement remains poorly understood,
due in part to the lack of quantitative description such as those carried out on the
behaviour of fibroblasts in culture (Abercrombie, Heaysman & Pegrum, 1970a-c).
In his cyto-embryological studies of amphibians, Sirakami (1963, 1972) proposed
a method for quantitating the circus movement and stated some features of this
movement. These included: the antagonistic relationship that exists between the
circus movement and cytokinesis, the height of the lobopodial wave and the velocity
of the movement which remain constant irrespective of cell size, and changes
propagating in cell surface which may regulate the pace of the circus movement or
the cleavage and developmental speed of the embryo.

The present study describes the circus movement of dissociated embryonic cells
of a fresh water teleost, Oryzias latipes. These cells were observed by time-lapse
cinemicrography in order to demonstrate their intrinsic and extrinsic factors for
the movement. Because of the relative transparency of the teleostean embryos, we
can also make use of in vivo studies on the behaviour of their cells (Trinkaus, 1973;
Kageyama, 1975).

MATERIALS AND METHODS

The developing eggs of the orange-red variety of the cyprinodont fish, Oryzias latipes
(Japanese Medaka) were used. Details, with respect to the nature of this fish, have been
previously reported (Yamamoto, 1967). The fertilized eggs attached in mass to the abdomen
of females were collected each morning. The eggs were transferred to dechlorinated tap
water and allowed to develop at room temperature (approximately 25 °C) until the initiation
of the experiments. Basically, the embryos were staged according to the chart of Matsui
(1949), with some of the modifications of Kageyama (1975). The eggs were washed and then
transferred into Ca**-free Yamamoto solution (0.75 % NaCl, 0.02 % KCl, and 0.002 % NaHCO³
at pH 7.3). In this solution eggs were dissected and blastoderms were mechanically dissociated
into their constituent cells with watchmaker’s forceps. Unless otherwise stated, cells were
collected by centrifugation at 200 g for 10 min, resuspended in rinse solution, collected again
and resuspended once more in Ca**+-free Yamamoto solution. Although these procedures
appear harsh, a suspension composed mostly of active individual cells was readily obtainable.
Immersion medium (0.5 ml) was added to each depression slide in which 0.05 ml of cell
suspension had been previously placed. In order to investigate the effects of pH on the
movement, cells were immersed in Ca**+-and NaHCO³-free Yamamoto solution of various
pH values containing Britton & Robinson’s universal buffer mixtures employing 5 mM acid
mixtures (phosphoric acid, boric acid and acetic acid) adjusted to the required pH by NaOH.
In some experiments the solution was buffered by Michaelis’ buffer (CH₂COOH-CH₃COONa
for pH 4, 5; KH₂PO₄-Na₂HPO₄ for pH 6, 7, 8; NH₄-NH₄Cl for pH 9, 10, 11). In the
temperature experiments the cell suspensions were placed in a glass cylinder which was
fixed to a Petri dish by means of paraffin wax. After cells were allowed to settle for 30 min
the temperature was changed by altering the water temperature within the Petri dish. The
temperature was frequently checked and maintained at constant values during the study.
Dissociated cells were immersed in Ca**+-free solutions of various strengths following the
methods described by Yamamoto (1941) in order to determine the osmotic effects on the
circus movement. For stock solution, 1 M NaCl 100 parts + 1 M KCl 2 parts was prepared.
This solution was diluted with a 0.002 % NaHCO³ solution at pH 7.3.

After 30-90-min incubations in the various types of test solutions maintained at 25 °C, the
cells showing circus movement were photographed on 16-mm film by a Nikon CFMA at an
interval between frames of 2 or 4 s. The films were analysed frame by frame with the use of
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an analytical projector, NAC Dynamic Frame, by tracing the paths of the lobopodial movement of each cell. As proposed by Sirakami (1972) the velocity of the circus movement can be represented as the linear velocity of displacement of the lobopodial wave front when the wave unilaterally propagates around the cell circumference in a plane relatively parallel to the substratum. Thus, the method for the estimation of the velocity in the present study (Fig. 1) is essentially the same as that reported in embryonic amphibian cells by Kageyama, Satoh & Sirakami (1975). The difference between these methods comes from the fact that the spherical shape of the motionless cell core is more distinct in amphibian cells than in teleostean cells. The velocity of the circus movement of each cell was expressed as a mean value obtained from analysing four or more frames of the film.

Fig. 1. The linear velocity of the movement is given as \( \frac{l}{t} \) if the front of the advancing wave moves from \( P_0 \) to \( P_t \) through the distance, \( l \), in a considerably short interval, \( t \).

RESULTS

Cell behaviour at different developmental stages

Cells from each embryo at different stages (morula to mid-gastrula) were dissociated in a deep depression slide and observed without washing or collecting by centrifugation. Immediately after cell dissociation, hyaline blebs or lobopodia bulge out rapidly and are inclined to rotate around the cell circumference. Formation of blebs, if any, seems delayed in dividing cells. These changes in cell surface immediately after cell dissociation increase in amplitude and in the number of cells involved according as development progresses. In a few minutes, most individual cells isolated from morula retract their blebs and become spherical. Synchronous cell divisions are frequently observed among these cells and a lobopodium sometimes spreads near the cleavage furrow on the surface of dividing cells. Blebs of cells from early blastula are also retracted. Some cells protrude blebs again, but these blebs remain rather stationary or intermittently propagate around the cell circumference. Cells from late blastula and gastrula show the surface activity more than cells from early blastula. After cells changing from a knotty or even a vermiform into a spherical shape (Fig. 10), blebs reappear on cells from late blastula or gastrula and continuously show various types of movements as follows: (1) blebbing, (2) jumping,
or hopping of blebs around the cell circumference, (3) the circus movement, which may be regarded as a successive jumping of blebs. A lobopodial wave of the circus movement usually propagates with a frequent reversal of its direction of propagation. In some cells, however, a lobopodial bleb was seen to pass unilaterally (2-48 times) around the entire cell circumference (Fig. 11). The time required for a lobopodium to propagate around the cell circumference is fairly constant for each revolution (Table 1).

<table>
<thead>
<tr>
<th>Cell</th>
<th>Diameter, approx., ( \mu \text{m} )</th>
<th>Revolutions</th>
<th>Direction</th>
<th>Average time/revolution*, s</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>15</td>
<td>48</td>
<td>Clockwise</td>
<td>63 ± 3</td>
</tr>
<tr>
<td>B</td>
<td>20</td>
<td>14</td>
<td>Counter clockwise</td>
<td>70 ± 5</td>
</tr>
<tr>
<td>C</td>
<td>20</td>
<td>17</td>
<td>Clockwise</td>
<td>66 ± 2</td>
</tr>
</tbody>
</table>

* The time required for a lobopodium to propagate around the cell circumference for one revolution (expressed as the mean ± standard deviation).

As shown in Fig. 2, the percentage of cells exhibiting a propagating bleb increases during late blastula and gastrula stage. Under longer observations, more blebs come to propagate around in dissociated cells from late blastula and gastrula, while blebs usually remain stationary in cells from early blastula. Therefore, the difference between early blastula and later stages is underestimated in Fig. 2. Dissociated cells from early blastula were observed until embryos of the same brood developed.
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Fig. 3. The velocity of the circus movement of several cells from late blastula at 25 °C against the indicated hours after cell dissociation.

Fig. 4. The velocity of the circus movement of cells at 25 °C is plotted against cell diameter. Some cells (■) from early blastula in an alkaline solution show the circus movement. ○, cells from late blastula; ●, cells from early gastrula. The regression line calculated is \( y = 0.04x + 0.10 \) (\( y \), the velocity of the circus movement, \( \mu m \text{ s}^{-1} \); \( x \), cell diameter, \( \mu m \)). A correlation coefficient of 0.57 (the number of cells measured = 63) is obtained.
to the late blastula stage. During these *in vitro* observations, there was no increase in the number of cells showing the circus movement. Generally speaking, cells remain healthy and active for about 3 h or more, and only a few cells flatten on the glass substratum.

The velocity of the circus movement decreases slightly over the course of 3 h (Fig. 3) with no significant difference in the velocity whether or not cells were washed free of yolk. However, the velocity slightly increases as the cell diameter increases (Fig. 4). Generally, the circus movement was determined on cells of diameter 15–20 μm in the experiments described below. It is interesting that some cells from morula or early blastula show the continuous circus movement in a hypotonic or an alkaline solution. Their velocity of the movement is slightly faster than those of cells from late blastula or gastrula in a neutral and isotonic solution, though their movements do not last as long and seem to be somewhat pathological.

**Effects of pH on the circus movement**

The motile activities and cell shapes were observed in the range between pH 3.0 and 12.0. Cells from late blastula or early gastrula were washed and immersed in the solution of various pH values. Initially, most cells are lobopodial. Cells in solution of pH between 6.0 and 9.0 show the active circus movement. There is no significant difference in the velocity of the movement in this range of pH (Fig. 5). Almost all cells in solution below pH 5.0 become spherical by retracting pseudopodia and begin to lose viability at pH 3.0 or 4.0. With increasing pH, some of these cells bulge out pseudopodia and show circus movement again. Immediately after immersion in solution of about pH 12, large hyaline lobes form and spread around the cell circumference. All of these cells become spherical, swell up and then show cytolysis, but at pH 10.0 some cells remain lobopodial. These results and the schematic representation of pH-dependency of cell shape are shown in Fig. 6. Filopodia, which can move but eventually disappear, are also induced by immersing cells in alkaline solutions. A few cells sometimes show both the filopodial movement and the circus movement.

**Effects of temperature on the circus movement**

Experiments were done with cells from late blastula or gastrula after washing and transfer to the Ca²⁺-free Yamamoto solution. Although the pH of the immersion media is also changed slightly by temperature, the velocity of the movement is not so affected by this minute change of pH as already shown in Fig. 5. Some cells from the same cell suspension were left at 25 °C and their velocities of movement were measured as controls. The results are shown in Table 2 and Fig. 7. The velocity of circus movement increases with temperature. After the temperature was shifted back to 25 °C, recovery of the initial velocity of movement was observed. The changes in the velocity of circus movement for the same cell were also determined by raising (Fig. 8A) and then lowering (Fig. 8B) temperature. The velocity of the movement in each cell fluctuates somewhat irregularly. Generally speaking, however, it changes as expected from Fig. 7. Biological zero of the circus movement (i.e. temperature
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Fig. 5. The velocity of the circus movement of cells from early gastrula at 25 °C plotted against pH value of medium from 6–10 adjusted with Britton & Robinson's universal buffer (○) or Michaelis' buffer (●).

Fig. 6. Schematic representation of pH-dependency of cell shape and the percentage of cells exhibiting a propagating bleb. Each plot represents the mean of duplicate cell counts after cells were immersed for 1 h in solutions of various pH values adjusted with Britton & Robinson’s universal buffer. O, cells from late blastula; ●, cells from early gastrula.

at which the movements are arrested by cold) may be below 5 °C, because cells showed the movement at this temperature when analysed at accelerated speed by the projector.

Effects of osmotic pressure on the circus movement

The osmotic pressure of the interior of Oryzias eggs was described by Yamamoto (1941) to be equivalent to 0.134 M or M/7.5 NaCl solution. Cell suspensions from late blastula or early gastrula were washed and transferred to the salt solutions of various concentrations as described in Materials and Methods (M/3, M/5, M/7, M/9, M/11, M/13, M/15 and M/17 salt solution). Cells placed in M/3 salt solution do not
Table 2. Effects of temperature on the velocity of circus movement, μm s⁻¹

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Velocity (μm s⁻¹)</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0.10 ± 0.03</td>
<td>11</td>
</tr>
<tr>
<td>10</td>
<td>0.37 ± 0.12</td>
<td>9</td>
</tr>
<tr>
<td>15</td>
<td>0.61 ± 0.19</td>
<td>9</td>
</tr>
<tr>
<td>20</td>
<td>0.53 ± 0.12</td>
<td>8</td>
</tr>
<tr>
<td>25</td>
<td>0.80 ± 0.27</td>
<td>62</td>
</tr>
<tr>
<td>30</td>
<td>1.31 ± 0.24</td>
<td>16</td>
</tr>
<tr>
<td>35</td>
<td>1.38 ± 0.31</td>
<td>7</td>
</tr>
</tbody>
</table>

The average velocity is expressed as the mean with standard deviation. The velocity of the control cells at 25 °C (see text) is in parentheses.

* N, the number of cells measured.

† The average velocity of the control cells of each experiment.

Fig. 7. The velocity of the circus movement of cells from early gastrula plotted against temperature. ●, cells at the indicated temperature; ○, cells at 25 °C as control from the same suspension.

show the typical circus movement but shrink, become crenated and sometimes show blebbing or jumping. In highly hypotonic solution such as M/15 and M/17, large hyaline blebs bulge out. Eventually the cells swell up and die. These effects of osmotic pressure on circus movement are reversed by exchanging the immersion fluid. The velocity of the movement does not seem to vary with osmotic pressure (Fig. 9). However, in hypotonic solutions, propagating blebs are larger than in hypertonic solutions.
**DISCUSSION**

When dissociated in an appropriate medium, embryonic cells show unique and remarkable movements. On embryonic teleostean cells in the present study, their lobopodia tend to rotate around the cell circumference. Other types of cell behaviour are observed rather in rare cases but immediately after cell dissociation, such as
peristaltic movement or 'tug' movement described by Sirakami (1963) and Kageyama et al. (1975).

Because it is a lobopodial activity of cell surfaces, the circus movement is not observed unless the immersion media are suitable for the formation of pseudopodia. For instance, the circus movement is reversibly prevented in a solution below pH 5 or in a hypertonic salt solution. Harris (1973) also stated that blebbing of fibroblasts was inhibited in a strongly hypertonic medium. These effects of pH and osmotic pressure on circus movement are essentially similar to those described by Holtfreter (1948). A highly hypotonic solution and a strong alkaline solution cause cells to form large hyaline lobopodia, even to cytolyse. In these solutions, some early blastula cells show continuous circus movement for a while, but this is not commonly observed in these cells while maintained in isotonic and neutral solutions. The velocity of circus movement does not change much if only a lobopodium is formed (Figs. 5, 9). However, Taylor (1962) reported that elevation of pH accelerated cell movements such as pinocytosis and the motion of cellular granules. The environmental conditions such as pH or osmotic pressure of the immersion fluid are suspected to affect the deformability of cell surface (Braatz-Schade, Haberey & Stockem, 1973; Weiss & Clement, 1969). We propose that the deformability of cell surface may be related to the mobility of cells in a complicated manner, which is discussed later.

The velocity of the circus movement is reduced by lowering temperature. The constancy of $Q_{10}$ with temperature or phase separation may be suggested in the present study (Table 2), but its relation to the temperature-dependency of the fluidity of membrane (Noonan & Burger, 1973) remains uncertain.

The importance of thiol-disulphide exchange in the circus movement is suggested (Fujinami, 1975). This was previously reported in amoeboid movement in Amoeba proteus, flagellar movement in Pandorina, and gliding movement in Oscillatoria (Abe, 1963), in zeiotic bubbling in murine tumour cells (Belkin & Hardy, 1961) or in cell division of sea-urchin eggs (Sakai & Dan, 1959). The circus movement is reversibly inhibited by cytochalasin B (Fujinami, 1975). These considerations lead us to relate circus movement to normal cytokinesis, which is another kind of cell movement. It is well known that the most active and irregularly shaped cells also assume a spherical shape at the time of cell division. Sirakami (1963) suggested that cytokinesis is antagonistic to all other forms of movement exercised in the life of a cell. He proposed that when a cell begins to divide, cytoplasmic motility is lost with surface movements being effectively suppressed. If cytokinesis is suppressed, the cell may perform other types of activity. Recently, Sanger (1974) suggested that cellular actin could be recycled to perform different functions (cytoskeletal, amoeboid movement and furrowing) during the cell cycle. If so, the difference between early blastula and later stages in Fig. 2 may be explained by the switching of this basic motile mechanism which is normally 'on' to cell division in cells of early blastula.

Cell surface changes of embryonic cells of Fundulus during the process of development have been described: increased deformability (Tickle & Trinkaus, 1973) and increased adhesivity (Trinkaus, 1963). We have observed that bleb formation, immediately after cell dissociation, appears to be associated with the embryonic
developmental stages. Cell surface changes tend to increase in amplitude and in the number of cells involved as development progresses, which is probably due to the increased deformability of cell surfaces during development. Because the environmental conditions in the present study are unsuitable for cells to adhere to other cells or to the glass substratum, we have not observed increased adhesivity, but dissociated cells of *Oryzias latipes* also show an increase in degree of reaggregation with respect to their stage of development in an appropriate medium (Yokoya, 1966). Johnson (1970) discussed the antagonistic relationship between 'limicola movements' and cell-substratum adhesions and Epperlein (1974) regarded the circus movement as a characteristic process of attachment of cells to the glass substratum. Therefore, the increased mobility of cells during their early developmental stages (Fig. 2) may depend on increased surface activities, which will appear as increased adhesivity if cells are observed in media suitable for adhesion. The increased surface activities might be based principally on increased deformability. In this respect, it is of interest that trypsin increases cell deformability (Weiss, 1966) and is able to promote the spreading of macrophages (Rabinovitch & DeStefano, 1973).

Holtfreter (1947, 1948) described in amphibian cells that the surface activity was restricted or reduced with progressive differentiation. Preliminary experiments in our laboratory show that no blebs form on liver cells from adult fish, when they are dissociated by trypsin while embryonic cells treated in the same manner show circus movement. Therefore, the cessation of lobopodial formation can be used as a convenient criterion of the onset of differentiative processes (Wilde, 1961).

The activities of pseudopodia or amoeboid movement during morphogenesis of embryos have been frequently reported in other species (Gustafson, 1964; Hamano, 1964; Wourns, 1972; Nakatsuji, 1974). Circus movement of deep cells *in vivo* was reported in the *Fundulus* blastoderm (Trinkaus, 1973) and in the *Oryzias* blastoderm (Kageyama, 1975), where their velocity was approximately the same as that observed in our study. The behaviour of the deep cells *in vivo* corresponds to our findings of isolated cells, but the role of the circus movement in morphogenesis remains to be explained.

**REFERENCES**


Circus movement of embryonic cells


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Fig. 10. The surface change of cells from late blastula (stage 12) immediately after cell dissociation and 1 and 4 min later, respectively, observed in the same field. The knotty-shaped or even vermiform cells become spherical owing to retracting blebs.

Fig. 11. A cell from early gastrula (stage 13) shows the stable circus movement in Ca²⁺-free Yamamoto solution at 25 °C (sequence of frames from a 16-mm ciné film exposed at intervals of 4 s). This unilaterally propagating lobopodium continuously passed around the entire cell circumference clockwise for 48 revolutions. The shape of the motionless cell core is deformed by blebbing and spreading of a lobopodial bleb if the lobopodium is large enough as compared with the cell. The scale mark represents 10 μm.