The Fish Biology Journal MEDAKA, 6: 1–5 (1994)

Oil droplet movement during ooplasmic segregation in the *Oryzias latipes* (medaka) fish egg

Bradley J. Catalone and Richard A. Fluck

Biology Department, Franklin and Marshall College, P.O. Box 3003, Lancaster, Pennsylvania 17604 -3003, USA.

(Received June 23, 1994)

Abstract During ooplasmic segregation in the medaka egg, one easily identifiable class of ooplasmic inclusions (oil droplets) segregate to the vegetal pole. In this study, we used time-lapse video microscopy to monitor closely the movements of these droplets in eggs oriented with either their animal pole or their vegetal pole uppermost with respect to gravity. After fertilization, the droplets moved first toward the animal pole and then toward the vegetal pole. In eggs oriented with their vegetal pole uppermost, oil droplets stopped moving toward the animal pole significantly sooner than droplets in eggs oriented with their animal pole uppermost. Moreover, the position of an oil droplet on a meridian connecting the animal pole and vegetal pole of the egg was a factor in determining when that droplet ceased moving toward the animal pole, with droplets nearer the animal pole stopping before droplets farther away from this pole. These results, together with others from our laboratory, suggest that at least three forces act upon oil droplets during ooplasmic segregation in the medaka egg: i) a buoyant force that causes droplets to float toward the top of the egg, ii) a force that restrains oil droplets from floating toward the top of the egg, and iii) a force that actively moves droplets toward the vegetal pole.

Introduction

The medaka egg consists of a thin (less than 30 μm thick) peripheral layer of ooplasm that is bounded on one side by the plasma membrane and on the other side by the yolk membrane, which separates the ooplasm from a large (diameter $\approx$ 1100 μm) yolk vacuole. The ooplasm of the egg contains dozens of oil droplets, ranging in diameter from less than 5 μm to more than 130 μm, that move toward the vegetal pole during ooplasmic segregation.

Previous studies have suggested that the movement of these droplets toward the vegetal pole is mediated by microtubules. In eggs treated with microtubule poisons—colchicine, demecolcine, or nocodazole—the droplets float to the top of the egg rather than move toward the vegetal pole (Abraham *et al.*, 1993a). Moreover, a dense network of microtubules is present in the ooplasm of the medaka egg during the time when the droples are moving toward the vegetal pole (Abraham *et al.*, 1993b). Because these droplets are large and easily identified, they may be particularly useful in the study of microtubule-based movement *in vivo*.

The objective of the present study was to monitor closely the movements of oil droplets at different latitudes from the animal pole of the egg. Specifically we sought to determine whether the movements of droplets near the animal pole of the egg differed from oil droplets more distant from the animal pole. A preliminary account of this study has been published (Catalone and Fluck, 1994).

Materials and Methods

Medaka eggs were fertilized *in vitro* and transferred to a square polystyrene spectrophotometer cuvette (volume $\approx$ 1 ml) filled with BSS (111 mM NaCl; 5.36 mM KCl; 1 mM $CaCl_2$; 0.6 mM $MgSO_4$; 5 mM HEPES, pH 7.3; Abraham *et al.*, 1993a). To hold an egg for the purpose of video recording, we pushed it gently, with either its animal pole or vegetal pole uppermost, onto a small mound of Dow Corning vacuum grease on the bottom of the cuvette. Eggs were mounted within 6–15 min after fertilization ($T_n \approx 0.10$; T_n, normalized time, where $T_n = 0$ is the time at which the egg is fertilized, and $T_n = 1.0$ is the time at which the first cell division begins), and videotaping continued until $T_n \approx 0.55$. Sibling eggs, fertilized at the same time, were monitored at regular intervals to determine the time at which cytokinesis began. The experiments were performed at room temperature (20–23°C).

Correspondence should be addressed to R.A.F.

To monitor oil droplet movement, we viewed the egg through a 20x objective lens (Nikon 20/0.4) mounted on a video camera that was connected to a time-lapse VCR and video monitor. Total magnification was 143x, and the field of view was approximately 1250 μm. During playback of the tape, we marked the position of each droplet at 2 min intervals on a plastic sheet placed over the screen of the monitor.

Because the image on the monitor was a two-dimensional projection of objects arrayed on the surface of a spherical object, we converted the position of each droplet into units of theta, that is degrees above or below the equator of the egg (Sakai, 1965), assuming the egg to be a sphere. Although the egg is not a sphere but rather an ellipsoid of revolution, the error made in assuming the egg to be a sphere is negligible (Sakai, 1965). From video prints of the eggs, we measured egg diameter (1180 ± 65 μm, $\bar{X}$ ± SD, n = 9) and defined the center of the egg by finding the point of intersection of radii perpendicular to each of two lines drawn tangent to the edge of the egg. We then calculated the angular position of each droplet along the animal-vegetal axis of the egg, designating the position of the equator as zero, positions between the equator and the animal pole as positive and positions between the equator and the vegetal pole as negative. We subsequently calculated the distance that each droplet moved during each interval.

The data summarized herein are based on an analysis of 59 droplets from eight eggs (four oriented with animal pole uppermost, four oriented with vegetal pole uppermost) collected from five female medaka. The diameter of the droplets ranged from 16 μm to 132 μm (62.1 ± 23.0 μm, $\bar{X}$ ± SD, n = 59).

Results

The movements of six oil droplets from a typical egg (Egg #3, oriented with its animal pole uppermost) are summarized in Figure 1. The movements of the droplets can be divided into at

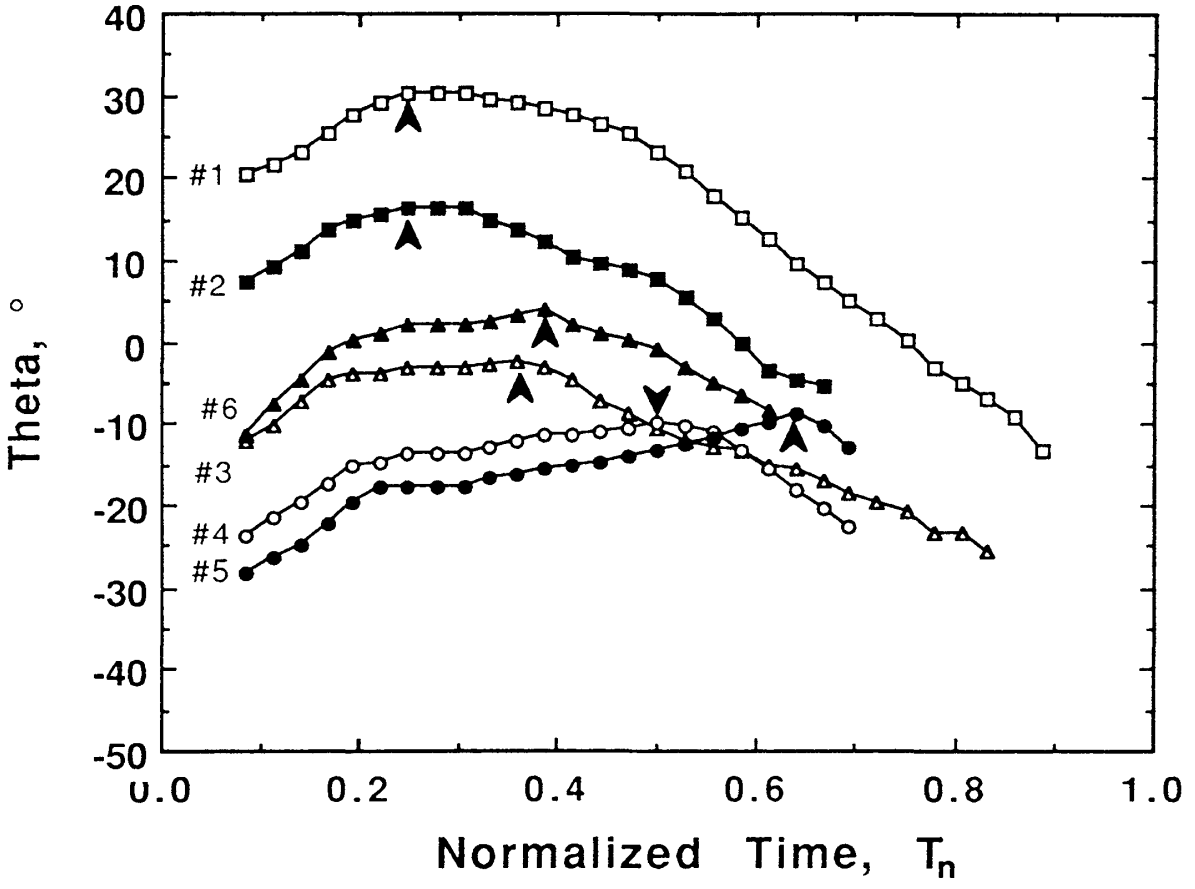

Fig. 1. Graphic summary of the movements of oil droplets in a single egg. The angular positions of six oil droplets (#1–6) from a single egg (Egg #3, which was oriented with its animal pole uppermost) are shown from T_n = 0.08 to T_n = 0.89). Initially all droplets moved toward the animal pole, rapidly at first and then more slowly. They then began to move toward the vegetal pole. The time at which each of the droplets came closest to the animal pole ("highest point time", arrowhead) was one of the parameters we used to compare the movements of droplets in eggs oriented with their animal pole uppermost vs. eggs with their vegetal pole uppermost.

least three phases. During the first phase, which was under way at $T_n = 0.08$ when we began videotaping and which lasted till $T_n \approx 0.19$, the oil droplets moved rapidly toward the animal pole of the egg. This movement corresponds to the "second contraction" of the ooplasm toward the animal pole of the egg (to distinguish it from the contraction that immediately follows fertilization of the egg; Abraham *et al.*, 1993a). During the second phase, the rate of movement of the droplets toward the animal pole slowed. This phase ended for each droplet when it reached its "highest point," the point at which it came closest to the animal pole. "Highest point time" (marked by arrowheads in Fig. 1) was the time at which this point was reached. The duration of the second phase was quite variable, with some droplets reaching their highest point by $T_n \approx 0.25$ (droplet #1) and others not reaching it until $T_n \approx 0.63$ (droplet #5). Finally, during the third phase of movement, the droplets moved toward the vegetal pole.

Most droplets (47) reached their highest point at $T_n = 0.13$–0.31 (Fig. 2). Oil droplets in eggs oriented with their vegetal pole uppermost reached their highest point significantly earlier than droplets in eggs with their animal pole uppermost (0.20 ± 0.08, $\bar{X} \pm SD$, $n = 30$; 0.27 ± 0.11, $n = 29$; $p = 0.0064$). Six of the seven droplets that reached their highest point later than $T_n = 0.3$ were in eggs oriented with their animal pole uppermost, while all five of the droplets that reached their highest point earlier than $T_n = 0.12$ were in eggs oriented with their vegetal pole uppermost.

The regression lines for eggs with animal pole uppermost, vegetal pole uppermost and for all eggs are also shown in Fig. 2. Two regression lines, one for eggs with their animal pole uppermost and another for eggs with their vegetal pole uppermost, fit the data significantly better ($p < 0.05$) than one line for all eggs because the y -intercepts of the lines for eggs with their animal pole uppermost differed significantly ($p = 0.0064$)

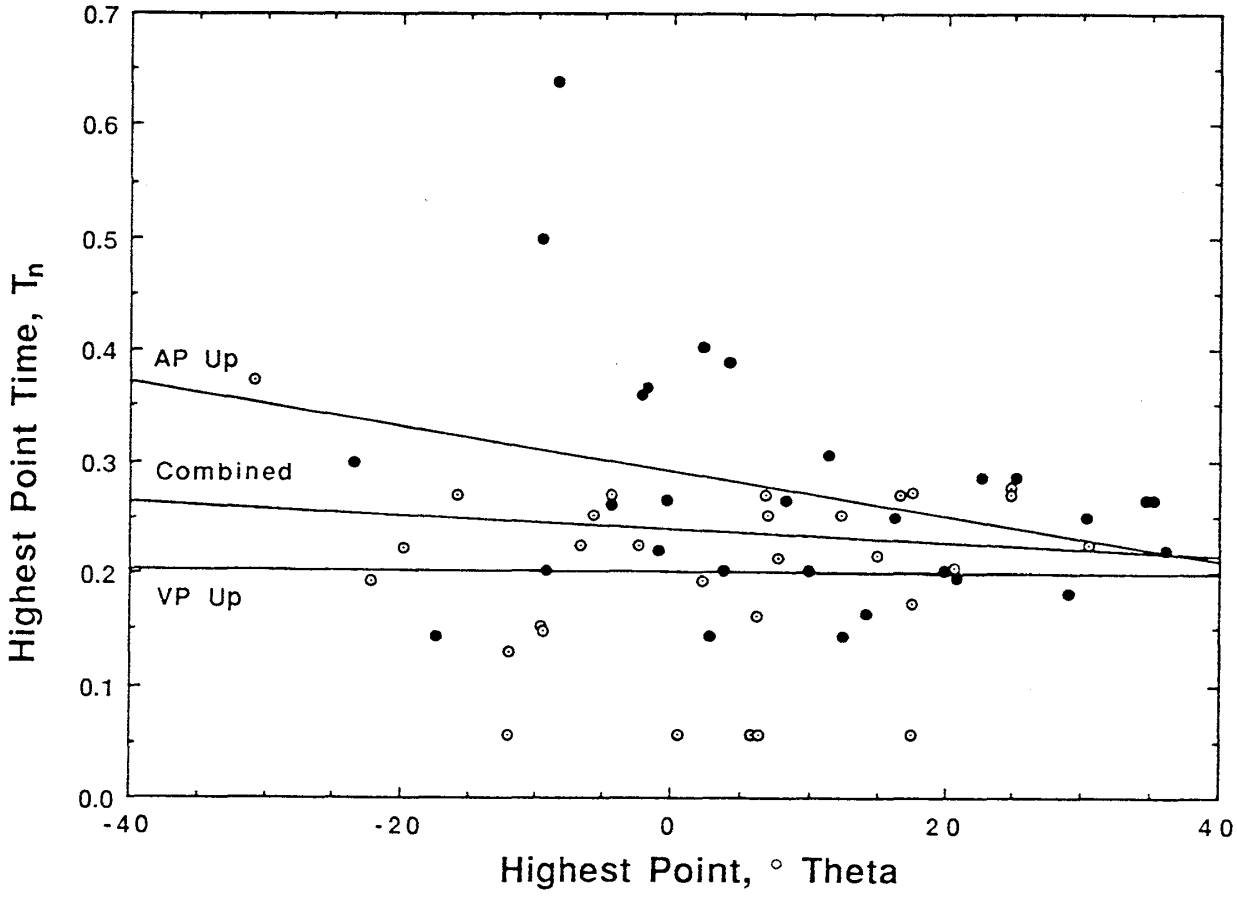

Fig. 2. The "highest point times" of oil droplets in eggs oriented with either their animal pole uppermost (●) or their vegetal pole uppermost (⊙). The "highest point times" of 59 droplets from about 30° below the equator to 35° above the equator are shown in this figure, in which positions below and above the equator are displayed as negative and positive values, respectively, on the abscissa. The regression lines of eggs with animal pole uppermost ($r^2 = 0.09$; $p = 0.1140$), vegetal pole uppermost ($r^2 = 0.000$; $p = 0.9455$) and for all eggs ($r^2 = 0.009$; $p = 0.4776$) are shown.

from those with their vegetal pole uppermost. The significant difference in y-intercepts is simply another expression of the fact that oil droplets reached their highest point earlier in eggs oriented with their vegetal pole uppermost. Drawing two lines with separate slopes did not give a significantly better fit than two parallel lines ($p = 0.22$). An analysis of covariance model in which we fit eight parallel lines to the data (one for each egg), showed that "highest point time" varied significantly with latitude, with oil droplets nearer the animal pole reaching their highest point significantly ($p = 0.0064$) sooner than oil droplets farther from the animal pole, regardless of the orientation of the egg. Using the slope of this relationship and assuming that a signal propagates from the animal pole to the vegetal pole of the egg, we computed a velocity of 1.02 μm/sec (95% confidence limits, 0.59 μm/sec–3.56 μm/sec).

Discussion

The results of the present study, taken together with others from our laboratory (Abraham *et al.*, 1993a, 1993b), suggest that at least three forces act upon oil droplets during ooplasmic segregation in the medaka egg.

A buoyant force causes droplets to float toward the top of the egg. Given the chemical (lipoidal) and physical (density) properties of the droplets, this seems to be a reasonable conclusion. Three observations of the behavior of the droplets in the egg support the existence of this force. First, oil droplets float to the top of eggs treated with microtubule poisons (Abraham *et al.*, 1993a). In the present study we found that a) the movement of oil droplets toward the animal pole during the second contraction was more pronounced in eggs oriented with their animal pole uppermost, and b) droplets reached their "highest point" sooner in eggs oriented with their vegetal pole uppermost. In eggs oriented with their vegetal pole uppermost, the buoyant force would tend to cause the droplets to move away from the animal pole and toward the vegetal pole of the egg. Thus, they would cease moving toward the animal pole (reach their highest point) sooner than droplets in eggs with their animal pole uppermost, in which the buoyant force would tend to cause the droplets to move toward the animal pole.

A force restrains oil droplets from floating toward the top of the egg. Several observations support the existence of this force. First, oil droplets are found throughout the ooplasm of unfertilized and just-fertilized eggs; they are apparently stable in their position for hours in unfertilized eggs, that is they do not float to the top of the egg. Second, during the second contraction of the ooplasm toward the animal pole, the oil droplets (and other inclusions as well) are pulled toward the animal pole (this study; see also *Abraham et al.*, 1993a). Third, in eggs treated with microtubule poisons, the droplets do not begin to float to the top of the egg until after the second contraction of the ooplasm toward the animal pole; their movements are apparently restrained before then (Abraham *et al.*, 1993a). In control eggs as well, the second contraction heralds the beginning of oil droplet movement, in this case toward the vegetal pole of the egg (Abraham *et al.*, 1993a). We suggest that this restraining force is exerted by a cytomatrix (Provance *et al.*, 1993) that can be disrupted by microtubule poisons and by a physiological regulator that acts soon after the second contraction, perhaps by solating the matrix (Janson and Taylor, 1993).

A force moves oil droplets toward the vegetal pole of the egg. In the present study this force became apparent after the droplets ceased moving toward the animal pole—reached their "highest point"—and subsequently began to move toward the vegetal pole, even when the animal pole was at the top of the egg. Two observations suggest that this force could involve microtubules. First, during the period when most oil droplets reached their "highest point", $T_n = 0.12–0.30$, a dense network of microtubules forms in the interpolar regions of the egg (Abraham *et al.*, 1993b). Second, in eggs treated with microtubule poisons, oil droplets do not move toward the vegetal pole but instead float to the top of the egg; in such poisoned eggs, the microtubule network is absent (Abraham *et al.*, 1993b). Oil droplets could associate with these microtubules via a microtubule-associated motor such as kinesin (Brady *et al.*, 1990; Schnapp *et al.*, 1992), which would also provide a means for moving the droplets along the microtubules. We are currently pursuing the question of the association of kinesin with the oil droplets and of the effects of microinjected anti-kinesin antibodies on the movement of oil droplets toward the vegetal pole of the egg.

The results of the present study also show that this force became effective near the animal pole first and then spread along the animal-vegetal axis of the egg. The velocity that we calculated for the spread of this force (1.02 μm/sec, 95% confidence

limits, 0.59 μm/sec–3.56 μm/sec) is too high for diffusion (Fluck *et al.,* 1994) and too low for the fast, reaction/diffusion calcium waves found in many cells (Jaffe, 1991). However, the velocity is within the range of the slow calcium and mechanical waves that accompany cytokinesis in the medaka egg (Fluck *et al.,* 1991). Such a wave might regulate microtubule polymerization as well as oil droplet movement, because microtubules appear near the animal pole of the fertilized egg before they appear elsewhere on the egg (Abraham, Miller, and Fluck, unpublished observations). We are currently pursuing the question of whether a calcium wave passes over the egg, from animal pole to vegetal pole, at Tn ≈ 0.12–0.30.

Acknowledgments

We thank Vivek Abraham for his interest in this project and for helping us improve the text of this paper, Valerie Stone and Tamika Webb for helping us improve the text of the paper, and Drs. Tim Hesterberg and David Lofsvold for their help with the statistical analysis of our data. This research was supported by NSF DCB-9017210 and NSF MCB-9316125.

References

Abraham, V.C., S. Gupta and R.A. Fluck (1993a) *Biol. Bull.,* **184:** 115–124.

Abraham, V.C., A.L. Miller, L.F. Jaffe and R.A. Fluck (1993b) *Biol. Bull.,* **185:** 305–306.

Brady, S.T., K.K. Pfister and G.S. Bloom (1990) *Proc. Natl. Acad. Sci. USA,* **87:** 1061–1065.

Catalone, B.J. and R.A. Fluck (1994) *J. PA. Acad. Sci.,* **67:** 173.

Fluck, R.A., A.L. Miller and L.F. Jaffe (1991) *J. Cell Biol.,* **115:** 1259–1265.

Fluck, R.A., A.L. Miller, V.C. Abraham and L.F. Jaffe (1994) *Biol. Bull.,* **186:** 254–262.

Jaffe L.F. (1991) *Proc. Natl. Acad. Sci. USA,* **88:** 9883–9887.

Janson, L.W. and D.L. Taylor (1993) *J. Cell Biol.,* **123:** 345–356.

Provance, D.W., Jr., A. McDowall, M. Marko and K. Luby-Phelps (1993) *J. Cell Sci.,* **106:** 565–578.

Sakai, T.Y. (1965) *Biol. Bull.,* **129:** 189–198.

Schnapp, B.J., T.S. Reese and R. Bechtold (1992) *J. Cell Biol.,* **119:** 389–399.