Reverse Bioconvection of *Chlamydomonas* in The Hyper-Density Medium

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Abstract

Chlamydomonas is known to form bioconvection as the result of its gravitactic swimming behavior. Several mechanisms have been discussed as the main cause of the preferential reorientation of the microorganisms with respect to gravity vector. In this study, we assessed the gravitactic orientation mechanism of *Chlamydomonas* by observing the bioconvective motion in the hyper-density medium containing Percoll. Observation of the bioconvection at the right angle to the convective motion (vertical observation) revealed that *Chlamydomonas* formed the reverse bioconvection in the hyper-density medium. Cells accumulated at the bottom of the chamber and then formed upward-moving plumes from the bottom accumulation. This reverse bioconvection indicates that the gravitactic orientation of *Chlamydomonas* is primarily due to the torque generated by the morphological fore-aft asymmetry of the cell. It is, therefore, highly likely that the orientation mechanism based on the drag-gravity model makes greater contribution in the gravitactic orientation of *Chlamydomonas* than other mechanisms, which include gravity sensing and gravity-buoyancy torque. ©2010 Jpn. Soc. Biol. Sci. Space; Article ID: 102403012

Introduction

Bioconvection brings an emergence of a macroscopic pattern as a result of the negative gravitactic migration of aquatic microorganisms. Many aquatic microorganisms swim preferentially upward and make top-heavy distribution within the water column. Since the average density of the organisms is higher than that of the surrounding water, an accumulation of the microorganisms leads to make falling blobs. During

downward movement, microorganisms come out of the blob and individually swim upwards. These movements of the opposite direction, downward due to gravity-induced settling and upward due to negative gravitactic swimming, would be collectively organized to form macroscopic patterns.

Negative gravitactic orientation requires the mechanisms responsible for generating the torque which causes the organisms to orient upward. Such mechanisms have been discussed in terms of either physical or physiological background. Gravitaxis of *Paramecium*, for example, can be explained both from the torque physically acting on the cell body even when being not alive (Fukui and Asai, 1980; Mogami *et al.*, 2001) and from the torque acting through the ciliary electro-motor coupling in association with cellular gravity sensing (Ooya *et al.*, 1992).

Organisms, in general, have some physical properties through which gravity acts to generate the torque. It is this physical torque that could bias the overall behavior even if the organisms have some physiological mechanisms controlling the orientation with respect to gravity. It is therefore plausible that orientation torques derived from the physical sources largely contribute to the development of gravitactic behavior, and thus to the formation of bioconvection pattern, especially for organisms without evident function of gravity sensing.

Although several mechanisms had been proposed for the sources of the gravitactic orientation torque, physical properties responsible for generating the torque are considered to be primarily caused by the asymmetry of either internal density or external morphology (Mogami *et al.*, 1995). These two mechanisms, summarized as gravity-buoyancy model and drag-gravity model, had been revealed to be functional in the swimming behavior of gravitactic microorganisms (Mogami *et al.*, 2001).

Gravity-buoyancy model, postulated by Verworn (1889) for the negative gravitaxis of *Paramecium*, is based on the density asymmetry within an organism. Since the center of gravity does not necessarily coincide with the center of buoyancy, posterior accumulation of the mass would result in upward orientation of the organisms, and anterior accumulation would result in downward orientation.

Drag-gravity model was postulated by Roberts (1970). In low Reynolds number condition, a geometrical fore-aft asymmetry (shape asymmetry) generates the torque as characterized by a dumb-bell with two spheres of unequal diameter but homogeneous density. According to Stokes' equation, the larger sphere of the dumb-bell sinks faster than the smaller sphere. The applicability of this model had been confirmed by scale-model experiments (Roberts, 1970).

Although the two orientation models predict a common sinusoidal change of the angular speed of the orientation with respect to the gravity vector, they can be distinguished by observing the orientation in the hyper-density medium. For the drag-gravity model, orientation torque is generated depending on the difference of

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the density between the cell and the external medium, while it is generated depending only on the density of the cell itself for the gravity-buoyancy model (Mogami *et al.*, 2001). Mogami *et al.* (2001) demonstrated that Ni^{2+} -immobilized cells of *Paramecium caudatum* orient downwards while floating upwards in a Percoll-containing hyper-density medium (the medium with higher density than that of the cell), but orient upwards while sinking in a hypo-density control medium (ordinary experimental medium with lower density than that of the cell). These findings indicate that the gravitactic orientation of *Paramecium* is primarily due to the torque generated by the morphological fore-aft asymmetry of the cell. In fact, Hirashima *et al.* (2003) demonstrated that *P. caudatum* showed a positive gravitactic migration in hyper-density.

Roberts (2006) reviewed the researches on the gravitactic mechanisms of *Chlamydomonas*, and concluded that the gravitaxis of this unicellular green alga is explicable in purely physical terms, in which orientation based on the drag-gravity model appears to play a main part of the gravitactic orientation of the cell body. If the conclusion by Roberts (2006) is true, we can expect for *Chlamydomonas* to swim downward in the hyper-density medium and to accumulate at the bottom of the chamber irrespective of the increased buoyancy greater than the gravitational force.

As mentioned above, bioconvection is caused by the accumulation of the gravitactically migrating organisms and the resultant density instability caused by the reverse density stratification of the medium. Upward-moving plumes would be formed from the accumulation which has lower average density than the above-located medium. During the upward movement, cells swim out of the plume, and the individual cells swim preferentially downward: the convection in the hyper-density medium would be expected to occur in the opposite direction to that in the hypo-density medium.

This "reverse bioconvection" is the target of this paper. It is a macroscopic evidence of the reversal of the convective movement; one is driven by physiological gravitactic swimming and the other by physical settling. Consecutive convective motion is possible only when these two components of movement function well.

If we can successfully induce the reverse convection of *Chlamydomonas* suspended in the hyper-density medium, it may prove the major role of the drag-gravity model for the gravitactic orientation of *Chlamydomonas*. In addition, the reverse convection may also present the evidence supporting the explanation of the initiation of the bioconvection from the density instability as the result of the uneven vertical distribution induced by the gravitactic migration.

Materials and Methods

Chlamydomonas reinhardtii (wild type, strain 137c, mt-) was prepared by a standard method described in Akiyama *et al.* (2005). Cells at the early stationary phases were spun down (300 $\times g$, 40 s) and resuspended in the

experimental medium, the basic composition of which was 1 mM KCl, 0.3 mM CaCl_2 , 0.2 mM EGTA (dissolved in 1 mM KOH), and 5 mM HEPES, pH = 7.9, adjusted by HCl (Yoshimura *et al.*, 2003). For hypo-density medium, above reagents were dissolved in water (MilliQ, Millipore Co. Ltd.). For hyper-density medium, 50 % (v/v) of water was replaced by Percoll (Sigma). The density of hypo- and hyper-density medium was 1.01 and $1.07 \times 10^3 \text{ kg m}^{-3}$, respectively, which are lower and higher than the average density of *C. reinhardtii* of $1.04 \times 10^3 \text{ kg m}^{-3}$ (Roberts, 2006).

The vertical pattern observations were done with a narrow chamber made of two sheets of slide glass (76 mm $\times$ 26 mm) separated by silicone rubber spacers of 1 mm thickness, which provided a 4 mm-deep slit for the formation of bioconvection patterns (Fig. 1). For video recordings, we used a high-vision camcorder (HDR-HC1, SONY, Tokyo, Japan), with which we can obtain images of 1920 pixels $\times$ 1080 pixels at a rate of 30 fps. For quantitative analyses, the images were converted to a stack of image files, each of which consists of 1920 pixels $\times$ 1080 pixels on a 256-level gray scale. Recordings were done using a flat light viewer (Hakuba Handy-viewer M35, Hakuba Photo Industry Co. Ltd., Tokyo, Japan) as a light source, which was placed on the backside of the chamber. Specimens were illuminated with red light through sharp-cut filters SC 64 ($\lambda > 640 \text{ nm}$) (Fuji Photo Film Co. Ltd., Tokyo, Japan), as *C. reinhardtii* is known to show no behavioral response to that red light (Matsuda *et al.*, 1998).

Space-time plots were made by a so called digital slit camera method (Mogami *et al.*, 2004), where a linear profile of image density was obtained from the region of interest in each sequential image usually consisting of a few lines of pixel, and pasted together in time sequence to give an image which has space and time dimensions.

Results

Previous studies on bioconvection have been largely based on the top-view observation/recording of the convection pattern developed in two dimensions. Top-views are suitable for the macroscopic analyses of the dynamic properties of the pattern formation, but not for the analyses of the local convective behavior of the cell population. In this study we introduced an experimental chamber which allows side-view observation of the vertical movement of the cell population (Fig. 1).

Fig. 2 shows a typical example of the side-view of the vertical movement of the population of *C. reinhardtii* suspended in the hypo-density medium. In a thin vertical layer of the medium (4 mm and 1 mm of depth and thickness, respectively), the cells were observed to accumulate at the top of the chamber, as shown in the sequential images of the response (a and b of Fig. 2A). Top accumulation is also shown in space time plot of the vertical portion of the cell suspension (Fig. 2B). Dark portion, at the top of the plot corresponding to the cell accumulation appears to increase with time.

Fig. 1. Schematic drawing of the chamber for the observation of vertical bioconvective motion. The chamber was made of two sheets of slide glass (sg) separated by silicone rubber spacers (ss) of 1 mm thickness, which provided a 4 mm-deep slit for the formation of bioconvection patterns.

Accumulated cells then formed settling blobs which grew to the downward plumes with a “bottle” shape; a plume with broad bottom and narrow neck (bottom image of Fig. 2A). Space time plot of the horizontal portion of the suspension (Fig. 2C) shows that formation of the downward plumes became stationary several minutes after the onset of the bioconvection, as indicated by parallel stripes at the lower part of the figure. Before the stationary formation, plumes moved laterally and fused with their neighbors, as indicated by “Y shape” connection of the stripes at the upper half of the space-time plot.

Fig. 3 shows a typical example observed on the population of *C. reinhardtii* suspended in the hyper-density medium. Sequential images of the movement demonstrate that *Chlamydomonas* accumulated at the bottom of the chamber (a and b of Fig. 3A). Bottom

Fig. 2. Vertical movement of the population of *C. reinhardtii* suspended in the hypo-density medium. Movement of the cell population in a thin vertical layer of the medium (4 mm and 1 mm of depth and thickness, respectively) were recorded at the right angle to the vertical side wall of the chamber. As the recordings were made under bright field (transmission) illumination, dark portions in each image correspond to the cell accumulation. A, sequential images of the recordings from the initial (a) to the time-passed states (f) of the movement. Linear regions indicated by faced triangles are the regions of interest on which space-time plots shown in B (open triangles) and C (filled triangles) were made. B, space-time plot of the vertical region showing the cell accumulation at the top of the chamber, which is represented by the growth of dark portion of the image. C, space-time plot of the horizontal region showing the lateral movement of the plume (dark vertical stripes) initiated after the top accumulation. Arrows a to f indicate the time corresponding to images shown in A. Double pointed arrow indicates the period in which the space-time plot (B) was prepared.

Fig. 3. Vertical movement of the population of *C. reinhardtii* suspended in the hyper-density medium. Details of the figure are the same as represented in Fig. 1. A, sequential images of the recordings from the initial (a) to the time-passed states (g) of the bioconvection. Linear regions indicated by faced triangles are the regions of interest on which space-time plots shown in B (open triangles) and C (filled triangles) were made. B, space-time plot of the vertical region showing the cell accumulation at the bottom of the chamber. C, space-time plot of the horizontal region showing the lateral movement of the plume. Arrows a to g indicate the time corresponding to images shown in A. Double pointed arrow indicates the period in which the space-time plot (B) was prepared.

accumulation is also shown in space time plot of the vertical portion (Fig. 3B), in which dark portion at the bottom of the chamber appears to increase with time. This result is in line with the positive gravitaxis explained solely by the drag-gravity model in the hyper-density medium. As the cells accumulated, unstable density distribution was created at the bottom of the chamber. Plumes were then formed from the bottom accumulation and moved upward (c to e in Fig. 3A). They appeared to be bottle-shaped similar to those formed in hypo-density medium, although they had diffuse boundary compared with those in hypo-density medium. The upward-moving plumes demonstrate that the bioconvection of *Chlamydomonas* in the hyper-density medium occurs in the opposite direction to that in the hypo-density medium.

This reverse bioconvection in the hyper-density medium, is not a complete reversal of that in the hypo-density medium. Space-time plot of the horizontal portion (Fig. 3C) shows that upward plumes were formed with longer delay than that in hypo-density medium. The plot also shows that plumes formed near the both edges of the chamber moved toward the center of the chamber. Plumes moved with uniting the neighboring plumes that

were already positioned nearer to the center. It should be noted that Fig. 3 shows the bioconvection formed in the cell suspension of twice the density of the suspension in Fig. 2. When we used the suspension of the same density as that used for the bioconvection in the hypo-density medium, the number of the upward plume decreased, and frequently only one plume was observed to be formed stationary near the center of the chamber.

We did not observe the accumulation of cells and the subsequent plume formation when *Chlamydomonas* was suspended in the isodensity-medium ($1.04 \times 10^3 \text{ kg m}^{-3}$).

Discussion

Side-view observation of the vertical movement of the population of *C. reinhardtii* revealed that *Chlamydomonas* in the hyper-density medium migrated downward and accumulated at the bottom of the chamber. Since buoyancy is greater than gravity in the hyper-density medium, the accumulation is likely to be caused by the preferential downward swimming. According to the physical mechanisms of gravitactic orientation of *C. reinhardtii*, both gravity-buoyancy model

and drag-gravity model predict the generation of upward-orienting torque in hypo-density medium. However, they predict the generation of torques directing oppositely in the hyper-density medium; upward by gravity-buoyancy model and downward by drag-gravity model. Therefore, the observation of the bottom accumulation of the cell demonstrates that the orientation mechanism based on the shape-asymmetry (drag-gravity model) functions dominantly.

In the hyper-density medium, upward-moving plumes were formed from the accumulation of the cell at the bottom of the chamber. The fact that the upward-moving plumes were maintained for several minutes suggests that reverse bioconvection occurred in the hyper-density medium while keeping the balance between the upward physical settling and downward gravitactic swimming.

Reverse bioconvection successfully induced in the hyper-density medium may support the explanation of the initiation of the bioconvection from the density instability as the result of the uneven vertical distribution caused by gravitactic migration. Initiation of bioconvection by the density instability was first theoretically analyzed in terms of Rayleigh-Taylor instability of the stratified fluid layer (Plesset and Winet, 1974; Plesset *et al.*, 1975). Childress *et al.* (1975) proposed a theoretical model which involves the upward swimming explicitly. Their analysis was based on the balance between the gravitactic swimming and the diffusive movement during settling, and certainly described indispensable aspects of bioconvective pattern formation. It should be noted that numerical simulation of convective motion on the basis of the model formulated by Childress *et al.* (1975) demonstrated the initiation of plumes and subsequent steady convective motion that is very similar to the result shown in this paper (Harashima *et al.*, 1988). Since the diffusive movement is highly dependent on the swimming activity of individual cells, changes in the convective motion could be assumed if the motile activity is reduced in the blob of the settling cells. Plausible effects of physiological changes, such as the lowered motility probably due to the hypoxic condition in the dense accumulation of the cell, could be incorporated in order to further verify the mechanisms of density instability-induced initiation of bioconvection.

The result presented in this study does not exclude the possibility of the generation of the orientation torque based on the density-asymmetry (gravity-buoyancy model). Kessler (1985) reported that immobilized biflagellate of related species to *Chlamydomonas* oriented upward in the medium of the same density as the cells themselves, which suggests the gravity-buoyancy model may function for the gravitactic orientation in the ordinary (hypo-density) media. The present result, however, demonstrates that the contribution of the orientation torque due to the density asymmetry is smaller than that to the shape asymmetry. The shape of *Chlamydomonas* resembles an ellipsoid (symmetry along the fore-aft axis) except for flagella, whereas the organelles such as chloroplasts are distributed asymmetrically along the fore-aft axis within the cell. Our findings verify that the asymmetry of the cell

body as a whole including flagella works more effectively in the bioconvection of *Chlamydomonas*.

Kessler (1985; 1986) demonstrated the gyrotactic property of *Chlamydomonas*, in which the hydrodynamic torque due to the spatial variation of the fluid velocity (vorticity) counteracts with gravitational torque. Accumulation of the cells in a beam on the axis of downwardly directed Poiseuille flow (Kessler, 1985) and the formation of a thin layer of phytoplankton in the coastal ocean (Durham *et al.*, 2009) are explained in terms of the gyrotactic property. It should be noticed that gravitational torque in the theory of gyrotaxis has been considered to be the torque generated due to the density asymmetry. Revision of the theory, therefore, might be required on the basis of the gravitational torque generated by the shape asymmetry which plays major role in the gravitaxis of *Chlamydomonas*.

The drag-gravity model predicts the torque which is generated in proportion to the difference between the densities of the cell and the surrounding medium (Mogami *et al.*, 2001). Although the density of the hypo- and hyper-density media in the present study were chosen to make the difference equal between the cell and each medium; $0.03 \times 10^3 \text{ kg m}^{-3}$, bioconvections in these different density media showed different aspects even beside the opposite direction of plume formation from the top or bottom accumulation: in the hyper-density medium plumes with diffuse boundary were formed with longer delay and moved toward the center of the chamber. The centripetal migration of the plumes was also observed in the plan view of the bioconvection formed in a wide-shallow chamber with a flat top and bottom (Akiyama *et al.*, 2005), where the pattern initially formed all over the chamber gradually migrated at the center of the chamber. This centripetal migration observed in the wide-shallow chamber was frequently followed by a sudden “burst” of the pattern spreading toward the periphery of the chamber. Such extraordinary behavior was not observed in the narrow chamber prepared in this study especially for the observation of the vertical convective motion.

Since the cells in the plume interact with each other through the surrounding medium, physical properties of the medium may affect the congestion within the plume. Percoll consists of nano-scale colloidal silica particles coated with polyvinylpyrrolidone. Although it has little effect on the osmosis and the bulk viscosity of the medium, the presence of the particle may change the local fluidity at the boundary on the cell surface. It might be, therefore, intriguing to examine the effect of medium viscosity/osmolarity on the delay of the emergence and the lateral mobility of plumes.

Evidence of physiological gravity sensing has not yet been accumulated for *Chlamydomonas*. Simple physical mechanisms, however, cannot fully explain the mutants with non- or less-gravitactic phenotypes (Yoshimura *et al.*, 2003). Reverse bioconvection presented above also does not exclude the possibility of the modulation of the swimming activity depending on the physiological gravity sensing.

For unicellular organisms, gravity-dependent behaviors are explained on the basis of a common cellular mechanism referred to as “statocyst hypothesis” (Machemer *et al.*, 1991; Machemer and Bräucker, 1992; Hemmersbach *et al.*, 1999), where the entire mass of a cell functions as a statolith which imposes mechanical stress on the mechanosensitive channels in the cell membrane. Although there is little evidence of the gravity-dependent regulation of the swimming behavior of *Chlamydomonas*, mechanosensitive channels were found to exist in the cell membrane (Yoshimura, 1996; 1998). In addition, TRP-channel gene homologs were found in the genome of *Chlamydomonas* (Martinac *et al.*, 2008). These lines of evidence of membrane mechanosensation may suggest that the statocyst hypothesis could also be applied to the basic mechanisms underlying gravitaxis of *Chlamydomonas*, which would also be inferred from the fact that the isolated mutants deficient in gravitaxis nevertheless displayed normal motility and had cell bodies of normal physical characteristics (Yoshimura, 2003).

Statocyst hypothesis, in which a whole cytoplasm is considered as a statolith, assumes the mechanosensation of the lower-most membrane of the cell depending on the difference of the hydraulic pressure between cytoplasm and external medium. It is therefore inferred that pressures with opposite signs would be induced at the membrane depending on the density of the external medium; positive pressure in the hypo-density medium and negative pressure in the hyper-density medium, respectively. Pressures acting oppositely on the membrane may induce different effect on the mechanosensation of the membrane.

Ooya *et al.* (1992) postulated a model of gravitactic reorientation of *Paramecium* based on the statocyst hypothesis of cellular gravisensation. Their model, referred to as “super helix model,” includes the electro-motor coupling of ciliary motility of *Paramecium* which allows paramecia to modulate the pitch angle of the swimming helix in response to the positive- and negative-going shift of the membrane potential on the basis of the unique fore-aft localization of depolarizing and hyperpolarizing mechanoreceptive channels (Ogura and Machemer, 1980). Computer simulation of the model had shown the upward swimming along a super helix trajectory (a large helix consisting of smaller helix) without any contribution of the physical reorientation mechanisms (Mogami and Baba, 1998).

If the shift of the membrane potential occurs in the opposite directions in the cells immersed in the hyper-density medium, super helix model would predict the downward swimming. In fact, Takeda *et al.* (2006) demonstrated that in the hyper-density medium *Paramecium* showed gravikinesis of opposite direction to that in the hypo-density medium. They pointed out the possibility that negative pressure generated in the hyper-density medium would decrease the open probability of the mechanosensitive channels. Lowered probability would cause the membrane potential to shift in the

opposite direction to that achieved by increasing the probability in response to the positive pressure generated in the hypo-density medium.

Although little is known about the gravity-dependent mechanoreception and the electro-motor coupling of the flagellar motility in *Chlamydomonas*, helical nature of the swimming trajectory suggests the possibility that physiological mechanisms similar to that discussed above for *Paramecium* would function as the physiological bias of the gravitactic orientation of *Chlamydomonas* in combination with the physical bias provided mainly from the fore-aft symmetry of the total morphology of the cell.

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