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Ciliary Movement of Sea-urchin Embryos

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Abstract

Living matter is constantly in motion: we human beings walk through the repeated contraction and relaxation of leg muscles; migratory fish swim long way also by the muscle contraction; a great number of unicellular and multicellular microorganisms swim about through the beating of miniature hairlike appendages called “cilia”. To address the unsolved question how cilia are regulated when these microorganisms explore their environment and are successful in life, we studied the ciliary movement of embryos and larvae of sea urchins by means of high-speed video micrography and by computerized analysis of ciliary beating at a high resolution. We found the four stages, E_1 through E_4, in the effective stroke with pauses intervening between one and the next. Serotonin, which has been demonstrated to increase the swimming speed of sea-urchin embryos, reduced reversibly the fluctuation and the length of period of E_4, and hence made the beating more stable and faster than before its application. Dopamine, which is known to decrease the swimming speed, increased the fluctuation and the period of E_4 antagonistically to serotonin and, in addition, prolonged the pause between E_1 and E_2 and reduced the rate of angular change in E_3. The potassium ion at higher concentrations than of standard seawater, which will depolarize the cell membrane and known to reduce the swimming speed, and to induce backward swimming in larvae of the pluteus and later stages, modified the beating in a manner similar to dopamine. Pauses between E_2 and E_3 often found in the pluteus stage were lengthened by serotonin and the potassium ion. These findings indicate that the internal motility machinery, i.e., the axoneme, can be regulated by modifying one or more of independently controllable stages of beating including E_1 through E_4 described in the present study.

Introduction

Living matter is constantly in motion: we human beings walk through the repeated contraction and relaxation of leg muscles; migratory fish swim long way also by the muscle contraction; a great number of unicellular and multicellular microorganisms swim about through the beating of miniature hairlike appendages called “cilia”. Cilia and flagella, similar hairlike appendages that are usually longer and less massive than cilia, are also found in our body; they are found in the ciliary epithelium of tracheal airway, the ventricle of the brain, the oviduct and as the tail of spermatozoon. The function of these organelles is undoubtedly important for individuals of ciliary dyskinesia often die at the early stage of development and, if lived longer, will be affected by heavy diseases and often infertile. Even in the healthy body, the activity of cilia and flagella are precisely regulated by the nervous system and under variable environmental conditions.

Sea-urchin embryos or larvae swim about through the beating of cilia, which cover the almost entire surface of the body (Fig. 1). They regulate the swimming behavior by gathering various keys from their environment; they control the vertical position in the water column changing the propulsion depending on the direction of swimming in the late stages of development (Mogami *et al.* 1988). They might also regulate the orientation against the physical torque, which has been demonstrated to alter in the nature of mechanical origin in the curse of development, heterogeneity of form or of mass distribution (Mogami *et al.* 2001). They also use cilia for filter feeding and regulate their activity responding to external stimuli, size or chemicals, and depending on internal states, hungry or stuffed (Strathmann, 1971). Thus, the cilia of sea urchin perform a variety of movement and regulation; in fact, they show at least five distinct states of activity (Mogami *et* *al.* 1991). The ciliated cells of sea urchins bear only one cilium of 9+2 axoneme per cell and are extraordinarily suitable for beating analysis when compared with those from other animals (see Fig. 2 and also Mogami *et al.* 1993). These are why we chose the cilia of sea urchin embryos as materials for the study of ciliary movement.

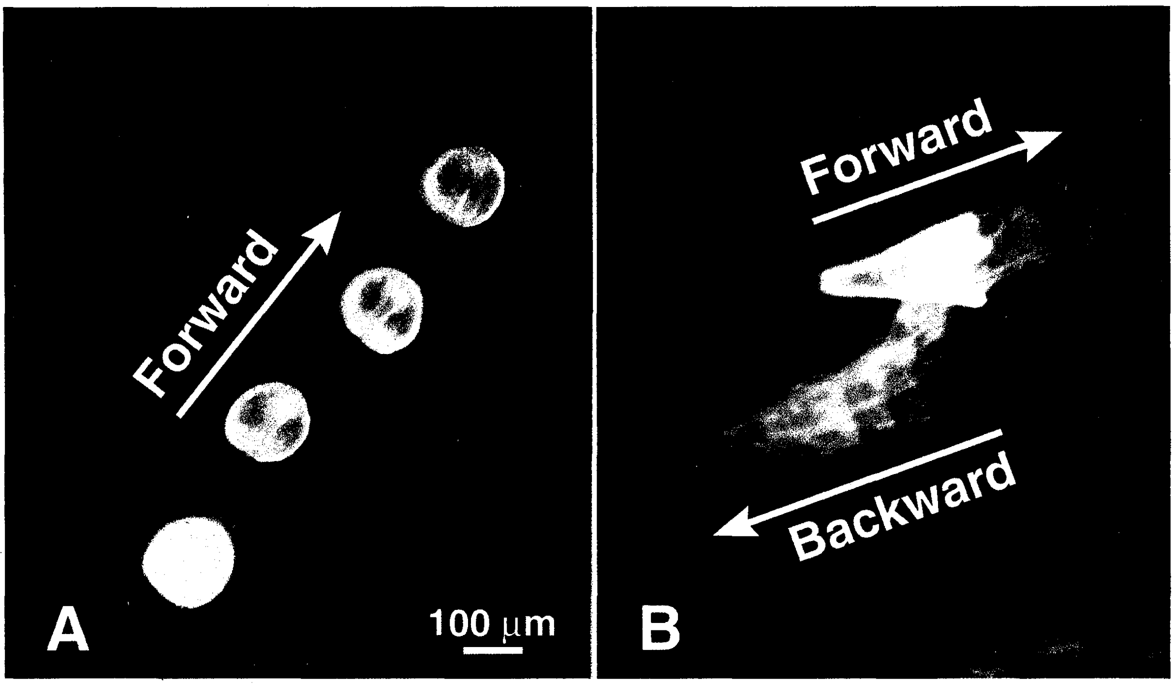

Fig. 1. Multiple-exposure photographs of swimming embryos of the sea urchin, *Hemicentrotus pulcherrimus*. A: gastrula stage; B: pluteus stage. Exposure intervals: 1 s (A) and 2 s (B); the brightest images are made by the first flash of stroboscopic exposure. 12 ℃.

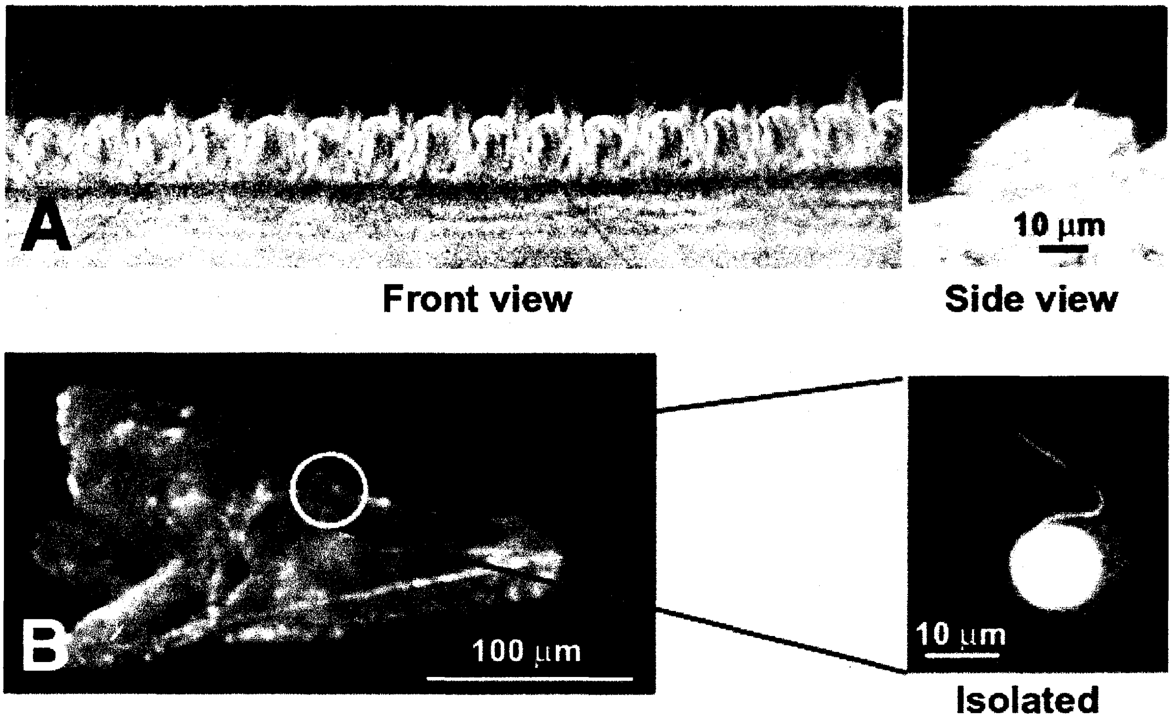

Fig. 2. Massive vs. single cilia. A: the lateral cilia of the gill of the mussel *Mytilus edulis*. B: the sea-urchin embryo cilia of the pluteus larva of *Hemicentrotus pulcherrimus*. Photos are from frames of high-speed cinematographs taken at 500 fps. The cell to the right of B was isolated from the ciliated epithelium of a pluteus larva in dissociation medium and was observed in ASW (for dissociation, see Mogami *et al.*, 1993). 23 ℃.

Several authors have demonstrated that the ciliary movement of sea urchins can be modified by the potassium ion (Markman, 1972), electrical stimulation (Baba, 1975; Baba and Mogami, 1987a), the calcium ionophore A23187 (Degawa *et al.* 1986) and pharmacological substances (Soliman, 1983; Mogami et al. 1992; Wada et al. 1997). Of these it intrigues us that dopamine increases the fluctuation of beat cycle as well as the duration of the cycle and serotonin reduces them antagonistically. It has been demonstrated that the cilia of early stages stop the motion short time at the middle of the effective stroke when stimulated electrically independently of the time of stimulation during one beat cycle; the time of pause fluctuates and the pause itself is found even before stimulation (Baba, 1975; Baba and Mogami, 1987a). The same phase dependent modification of beating and the fluctuation of beating have been described with the large abfrontal cilia of *Mytilus* gill by Baba (1979). In the present study, we found that the modification of beating by dopamine, serotonin and increased concentrations of the potassium ion is due to the phase dependent regulation of the length and rate of four distinct stages and the length of pauses between them.

Materials and Methods

Embryos of the sea urchin, *Hemicentrotus pulcherrimus,* were reared to the stage of gastrula through pluteus at 17 ℃. The embryos in artificial seawater (ASW) were placed in the specially designed chamber made of a slide glass with two depression holes on both sides of a cover slip placed on the embryos as shown in Fig. 2. The movement of a cilium just brought in focus (inset of Fig. 2) was recorded with a Kodak HC1000 high-speed video camera through phase-contrast optics and with xenon flashes operated on a laboratory-designed power supply (Baba and Mogami, 1987b; Miyake *et al.* 1998). ASW containing 10^{-5} M dopamine, 10^{-5} M 5-hydroxytryptamine (5-HT or serotonin), or K^+ at increased concentrations (up to 160 mM as compared with 10 mM of the standard ASW) was perfused through the chamber; the movement was recorded in one of these experimental solutions and in ASW later again. The time course of the shear angle or angular direction of the cilium, as defined in Fig. 3, was measured by a software for automatic tracking of the cilium along its length, Bohboh (BohbohSoft, Tokyo) as shown in Fig. 4. The framing rate of the high-speed camera was 1000 fps so that the movement could be analyzed at a resolution of 1 ms, which revealed the characteristic features of distinct four stages of beating.

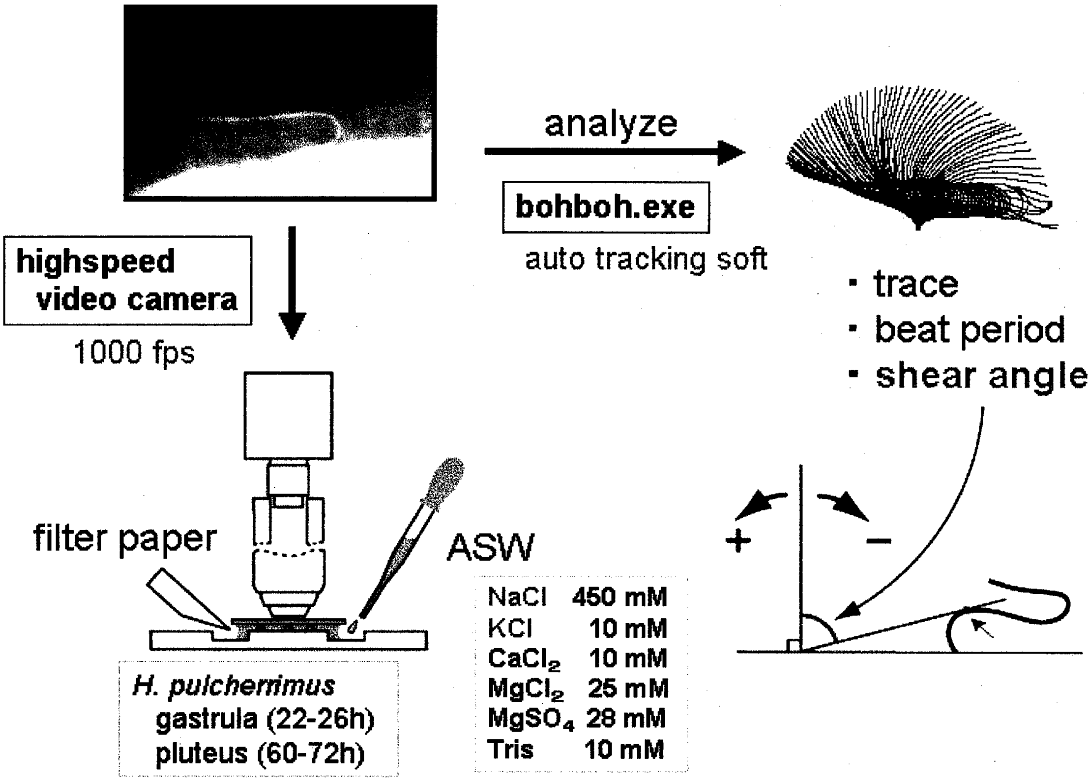

Fig. 3. High-time resolution analysis of ciliary beating in terms of the shear angle. This diagram illustrates the experimental chamber, microscope, analysis with an auto tracking software, and the definition of the shear angle.

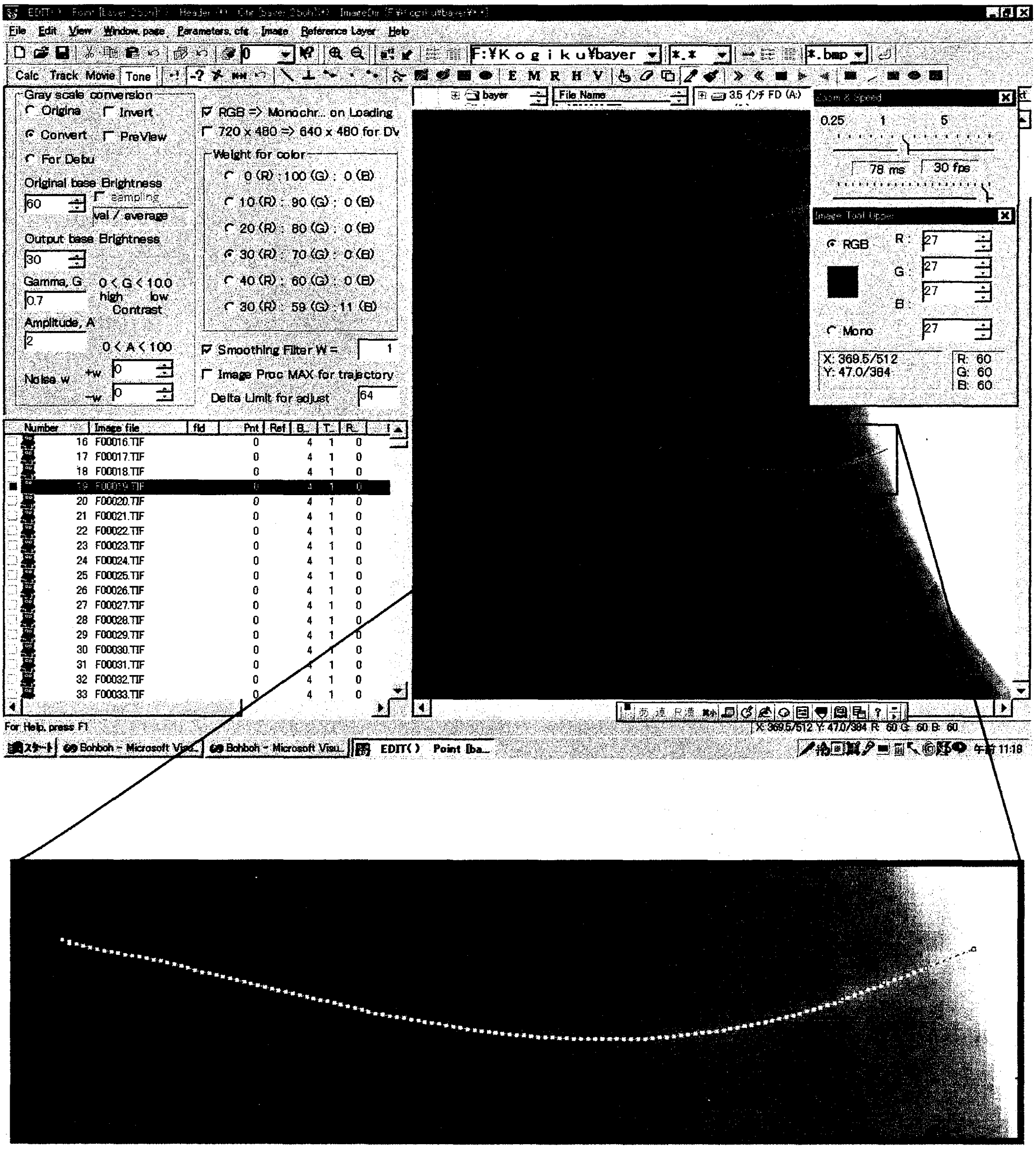

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Fig. 4. An auto tracking software, Bohboh.exe. A cilium of a sea-urchin embryo is ready for tracking (upper) and the tracking has been finished with some 130 points being determined along the cilium (lower).

Results and Discussion

The beating of a cilium was fluctuated in the length of time even in the standard seawater particularly in the late stage of the effective stroke. From the analysis of beating of the cilium in experimental solutions, we identified at least four distinct stages, E_1 through E_4 in the effective stroke in terms of phase, fluctuation, length and rate (Fig. 5). As summarized in this figure, the modification of beating is phase dependent: the pause between E_1 and E_2 is elongated by dopamine and high K^+; E_2 is little affected by all tested; the pause between E_2 and E_3 is elongated a little by serotonin and high K^+; the rate of angular change in E_3 is reduced by dopamine and high K^+; E_4 often with small pauses is greatly prolonged by dopamine and high K^+. These findings indicate that different components of the axonemes may work for the motility at different phases of beating and have a specificity to different conditions, respectively. The separation of these components in terms of regulatory proteins or putative phosphorylation remained to be studied.

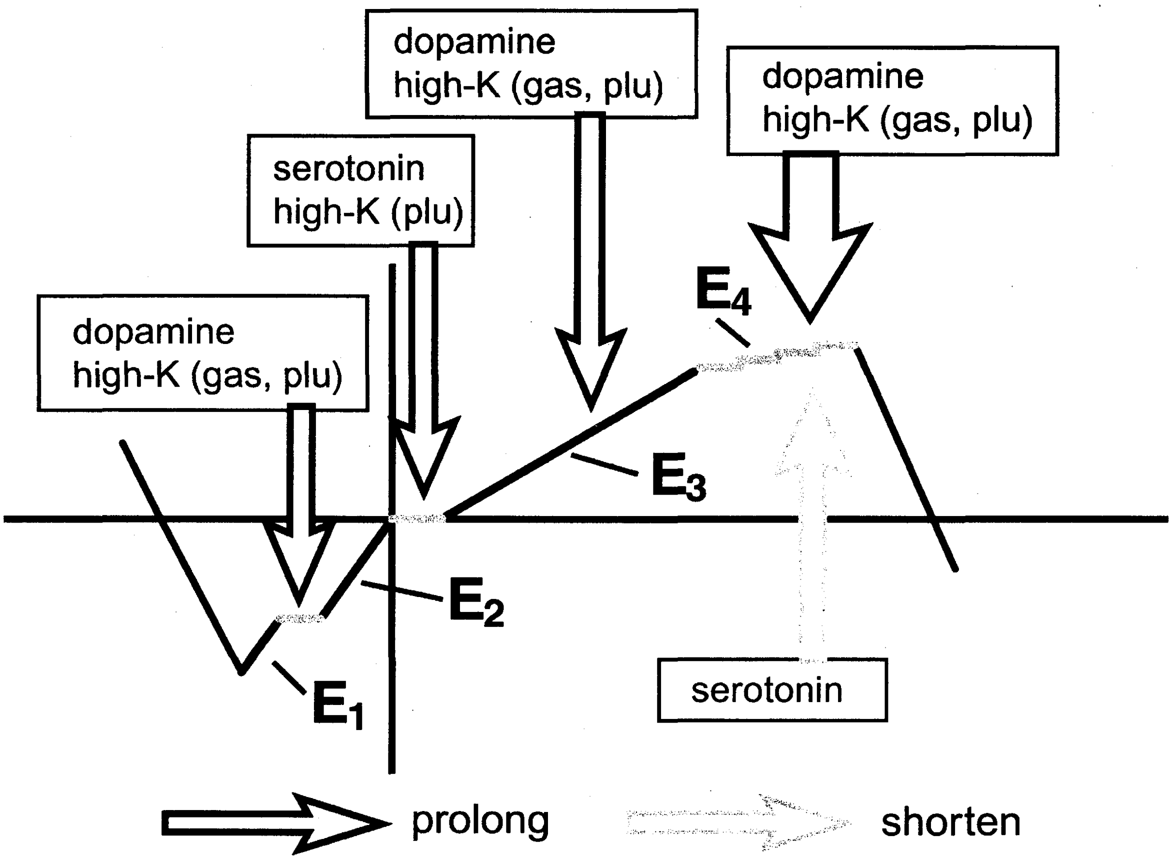

Fig. 5. Four stages E_1 through E_4 in the effective stroke of embryo cilia of *Hemicentrotus pulcherrimus* and intervening pauses between them. The time course of the shear angle measured at 5 μm from the base is drawn schematically. gas: gastrula; plu: pluteus.

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