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Calcium Control of Ciliary Reversal in Ionophore-treated and ATP-reactivated Comb Plates of Ctenophores

SHOGO NAKAMURA and SIDNEY L. TAMM

Boston University Marine Program, Marine Biological Laboratory, Woods Hole, Massachusetts 02543. Dr. Nakamura's present address is Department of Biology, Faculty of Science, Toyama University, Toyama 930, Japan.

ABSTRACT Previous work showed that ctenophore larvae swim backwards in high-KCl seawater, due to a 180° reversal in the direction of effective stroke of their ciliary comb plates (Tamm, S. L., and S. Tamm, 1981, *J. Cell Biol.*, 89: 495–509). Ion substitution and blocking experiments indicated that this response is Ca^{2+} dependent, but comb plate cells are innervated and presumably under nervous control. To determine whether Ca^{2+} is directly involved in activating the ciliary reversal mechanism and/or is required for synaptic triggering of the response, we (*a*) determined the effects of ionophore A23187 and Ca^{2+} on the beat direction of isolated nerve-free comb plates dissociated from larvae by hypotonic, divalent cation-free medium, and (*b*) used permeabilized ATP-reactivated models of comb plates to test motile responses to known concentrations of free Ca^{2+}. We found that 5 μM A23187 and 10 mM Ca^{2+} induced dissociated comb plate cells to beat in the reverse direction and to swim counterclockwise in circular paths instead of in the normal clockwise direction. Detergent/glycerol-extracted comb plates beat actively in the presence of ATP, and reactivation was reversibly inhibited by micromolar concentrations of vanadate. Free Ca^{2+} concentrations $>10^{-6}$ M caused reversal in direction of the effective stroke but no significant increase in beat frequency. These results show that ciliary reversal in ctenophores, like that in protozoa, is activated by an increase in intracellular free Ca^{2+} ions. This allows the unique experimental advantages of ctenophore comb plate cilia to be used for future studies on the site and mechanism of action of Ca^{2+} in the regulation of ciliary motion.

Calcium ions play a key role in the regulation of ciliary and flagellar activity during a variety of behavioral responses of protists and metazoans. Increases in the internal concentration of free Ca^{2+} (usually to 10^{-6} M) are responsible for changes in the direction of the ciliary effective stroke in *Paramecium* (7, 17, 18), reversal of the direction of flagellar wave propagation in trypanosomes (9), alteration of flagellar waveform in sperm and algae (2, 4, 5, 10), activation of beating of *Mytilus* gill abfrontal cilia (21) and ctenophore macrocilia (S. L. Tamm, manuscript in preparation), and arrest of beating in mussel gill lateral cilia (16, 20, 31, 32), *Chlamydomonas* flagella (11), and sea urchin sperm (8).

We previously reported one of the first clear examples of ciliary reversal in metazoans (23, 29). Using high-speed cinemicrography of larvae of the ctenophore *Pleurobrachia*, we showed that high K^+ concentrations cause a 180° reversal in the beat direction of ciliary comb plates, resulting in backward swimming. We took advantage of the built-in ultrastructural markers of comb plate cilia to show that neither the ring of outer doublets nor the central pair microtubules rotates during ciliary reversal (29). This finding indicated that the structural polarity of the axoneme does not uniquely determine the direction of the asymmetric beat cycle and that the orientation of the central pair does not control the direction of the ciliary effective stroke.

Ion substitution and blocking experiments with artificial seawaters (ASWs)[1] and divalent cations indicated that ciliary reversal in ctenophores is a Ca^{2+}-dependent response (29). However, because comb plate cells are innervated and ciliary

[1] *Abbreviations used in this paper*: ASW, artificial seawater; CMF, Ca^{2+}–Mg^{2+} free; RS, reactivation solution; WS, wash solution.

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reversal in adult ctenophores is presumed to be under nervous control (15, 24, 25, 28), it was not clear whether Ca^{2+} is required for synaptic triggering of reversal, activation of the axonemal reversal mechanism itself, or both processes.

In this study we tested whether Ca^{2+} is directly involved in the ciliary reversal response of ctenophore comb plates by determining the effects of calcium ionophore A23187 on the beat direction of dissociated comb plate cells and by using permeabilized ATP-reactivated models to analyze motile responses to known concentrations of free Ca^{2+} ions. We show that ciliary reversal of comb plates, like that of protozoan cilia, is caused by an increase in internal free Ca^{2+} concentration. A preliminary report of some of these results appeared previously (27).

MATERIALS AND METHODS

Ctenophore Larvae

Sexually mature *Mnemiopsis leidyi* were dipped carefully from Great Harbor, Woods Hole, MA, during summer 1983 and placed singly into large glass bowls of seawater at ambient ocean temperatures. By the next morning, the ctenophores (hermaphroditic, self-fertilizing) had spawned, leaving large numbers of eggs in early cleavage stages at the bottom of the bowls. The adult ctenophores and overlying seawater were removed, and the bowls were refilled with fresh seawater to allow development into free-swimming cydippid larvae. 2–4-d-old larvae were typically used for most experiments.

To obtain rapidly many motile larvae, the contents of the bowls were centrifuged in 100-ml oil centrifuge tubes (Corning 8200; Corning Medical and Scientific Corning Glass Works, Medfield, MA) at low speed for 4 min in an IEC Clinical centrifuge (International Equipment Co., Needham Heights, MA) without a protective shield. Larvae accumulated in the narrow stem of the tubes and were pipetted into a glass depression. This method provided hundreds of concentrated cydippid larvae in excellent condition. Aliquots of swimming larvae were transferred to test solutions with a braking pipette.

Reagents

Adenosine-5-diphosphate (ADP, trilithium salt), adenosine-5-triphosphate (ATP, dipotassium salt), and A23187 (calcium ionophore) were obtained from Calbiochem-Behring Corp. (La Jolla, CA); ethyleneglycol-bis-(β-amino-ethyl ether) *N,N'*-tetraacetic acid (EGTA), piperazine-*N-N'*-bis(2-ethane sulfonic acid) (PIPES), polyethylene glycol (20,000 mol wt), dithiothreitol, *N*-ethyl maleimide, mersalyl acid, and *p*-hydroxymecuribenzoate from Sigma Chemical Co. (St. Louis, MO); and sodium vanadate from Fisher Scientific Co. (Pittsburgh, PA).

Ionophore Experiments

SOLUTIONS: Ca^{2+}-free and Ca^{2+}–Mg^{2+}-free (CMF) ASWs were made according to Cavanaugh, G.M., editor, 1956, Formulae and Methods VI. MBL Chemical Room. Calcium ionophore A23187 was dissolved as 10 mM stock in methanol or in dimethylsulfoxide/ethanol (1:1) and diluted 1:1,000 or 1:2,000 in CMF–ASW to give final concentrations of 10 or 5 μM A23187. Calcium was added to give final Ca^{2+} concentrations of 0.5, 1, 2, 5, or 10 mM.

INTACT LARVAE: Larvae were pipetted into an excess volume of calcium free–ASW for 4–6 min to wash out residual Ca^{2+}. Larvae swam forward and appeared normal in this medium. Batches of larvae were then transferred into CMF–ASW containing 5 or 10 μM A23187 with varying concentrations of Ca^{2+}.

DISSOCIATED COMB PLATE CELLS: We used two methods to obtain isolated living comb plate cells from larvae: 4°C treatment in seawater overnight, or hypotonic divalent cation-free "dissociation solution," consisting of 150 mM KCl, 1 mM EGTA, 2% polyethylene glycol, 30 mM PIPES, pH 7.0, for 5–10 min at room temperature. Both methods caused larvae to shrink and disintegrate into single cells or small groups of cells with actively beating comb plates. Isolated comb plate cells swam in circular paths and remained motile for at least several hours. For convenience, we routinely used the latter method.

To test the effects of A23187 and Ca^{2+} on the motility of dissociated comb plate cells, 10 μl of comb plate cells was initially pipetted into 0.5 ml CMF–ASW containing A23187 ± Ca^{2+} for 3–4 min. 10-μl samples of cells were then added to 50 μl fresh test solution on a microscope slide, and the preparation was sealed with a Vaseline-edged coverslip for video recording.

Reactivated Comb Plate Models

Larvae were extracted in 0.005% Triton-X 100, 10% glycerol, 2% polyethylene glycol, 150 mM KCl, 2.5 mM $MgCl_2$, 1 mM EGTA, 30 mM PIPES, pH 6.9 for 2 min at 21–23°C. 10 μl of larvae in this solution was pipetted directly into 50 μl of wash or reactivation solution on a microscope slide, gently mixed, and covered with a Vaseline-edged coverslip. Reactivation solution (RS)[1] consisted of 2 mM ATP, 2.5 mM $MgCl_2$, 2% polyethylene glycol, 150 mM KCl, 1 mM EGTA, 1 mM dithiothreitol, 30 mM PIPES, pH 6.9. Wash solution (WS)[1] was identical to RS but lacked ATP. To test effects of Ca^{2+} on reactivated motility, Ca^{2+} was added to RS, the pH was determined, and free Ca^{2+} concentrations were calculated according to Salmon and Segall (19).

Light Microscopy and Analysis

Motility of comb plates was observed through a Zeiss Universal microscope with phase-contract optics and recorded with a stop-motion shutter video camera (Tritronics, Inc., Burbank, CA) at 60 fields/s and 2 ms exposure on a video cassette recorder allowing still-field playback (GYYR Products, Anaheim, CA). Profiles of beating comb plates in successive video fields were traced on cellulose acetate directly from the monitor (Panasonic WV-5410, Panasonic Co., Secaucus, NJ) and were used to determine beat form and direction. We obtained beat frequency from the tracings or by counting the number of fields per beat cycle directly from the monitor.

We took 35-mm films (Kodak 2415, Technical Pan) through a Zeiss RA microscope with phase-contrast optics, using an Olympus OM-2 camera and an Olympus T32 flash tube inserted in the illumination path.

Electron Microscopy

Living larvae were treated with 2.5% glutaraldehyde, 0.14 M NaCl, 0.2 M Na cacodylate, pH 7.8 at room temperature, then centrifuged for 30 s in a microfuge (model B; Beckman Instruments, Inc., Palo Alto, CA). The pellet was fixed for 1 h, then washed in 0.3 M NaCl, 0.2 M Na cacodylate buffer (pH 7.8) for 30 min, postfixed in 1% osmium tetroxide, 0.375 M NaCl, 0.1 M Na cacodylate for 30 min at room temperature, washed briefly in distilled water, dehydrated in acetone, and embedded in Araldite.

To fix ATP-reactivated comb plate models, we first checked reactivation of larvae in a depression slide under a dissecting microscope. Glutaraldehyde fixative (same as above) was then added after ~1 min in RS. Fixed models were pelleted in a microfuge and processed as described above.

Thin sections were cut on a Reichert OmU2 ultramicrotome with a diamond knife, placed on Formvar-coated grids, stained with uranyl and lead salts, and examined with a Zeiss 10CA electron microscope at 80 kV.

RESULTS

General Morphology of Cydippid Larvae

Cydippid larvae of *Mnemiopsis* closely resemble adult ctenophores of the order Cydippida (i.e., *Pleurobrachia*; see reference 29). 2–4-d-old larvae are 400–500 μm long and have a ciliary system that is disproportionately large relative to body size (Fig. 1). Each of the eight rows of ciliary comb plates contains about six plates at this stage. A comb plate consists of several hundred cilia, 125–150 μm long, which beat together as a unit (Fig. 1). At rest, comb plates are bent at the base and lie close to the body surface pointing toward the mouth. The planar beat cycle normally begins with an effective stroke, consisting of a rapid swing of the plate in the aboral direction which propels the animal mouth first (Fig. 1). In the recovery stoke, plates unroll in the oral direction by propagating a bend distally.

Effects of High-Ca^{2+} ASW on Intact Larvae

Increasing the Ca^{2+} concentration of sea water to 25–50 mM caused larvae to swim backwards slowly for several minutes with a high beat frequency. Larvae soon stopped moving, but beat frequency remained high. After 5–10 min, beat direction renormalized, beat frequency declined, and many larvae resumed forward swimming.

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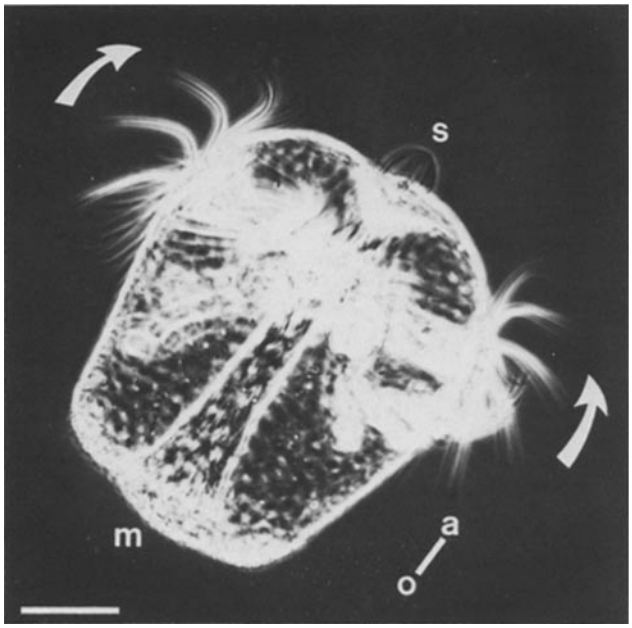

FIGURE 1 *Mnemiopsis* larva swimming forward in seawater. The statocyst (s) and mouth (m) define the aboral-oral axis (a—o) of the body. Two rows of comb plates are caught in profile on either side of the larva: in the more focused row on each side, plates are performing effective strokes (arrows) toward the aboral end in an aboral-oral sequence (antiplectic metachronal coordination). On the left, plates in the inactive row lie at rest pointing toward the mouth. Electronic flash, phase-contrast optics. Bar, 100 μm. × 130.

Effects of Calcium Ionophore on Intact Larvae

In CMF–ASW with 5 or 10 μM A23187 and 0, 0.5, or 1 mM Ca^{2+}, larvae swam forward for several minutes but then shrank and dissociated into cells or groups of cells with beating comb plates. Increasing the Ca^{2+} concentration to 2 mM resulted in both forward and backward swimming, with less tendency of the larvae to disintegrate. At 5 or 10 mM added Ca^{2+}, larvae consistently swam slowly backwards with a high beat frequency for 5–10 min and remained intact for longer periods.

Effects of Calcium Ionophore on Dissociated Comb Plate Cells

To determine whether ionophore-mediated ciliary reversal is triggered directly by Ca^{2+} influx into the comb plate cells themselves (regardless of whether or not Ca^{2+} activates neurociliary synapses which may trigger reversal) we examined the effects of A23187 on the beat direction of isolated comb plate cells dissociated from the larvae.

Comb plate cells isolated from larvae by dissociation solution or cold treatment appeared similar by phase contrast (Fig. 2, *A* and *B*) and differential interference-contrast microscopy. A single comb plate arose from one or several epithelial cells at this stage of larval development. Dissociated comb plate cells were roughly spherical and were free of any other cell types or axonlike processes. Because nerve cells and/or axons, if present, should be recognizable by light microscopy, the isolated comb plate cells are considered to be free of intact, functional nervous elements. The plates themselves retained the sharp, orally directed bend at the base characteristic of resting plates in intact larvae (Figs. 1 and 2). This asymmetry provided a marker for the oral–aboral axis

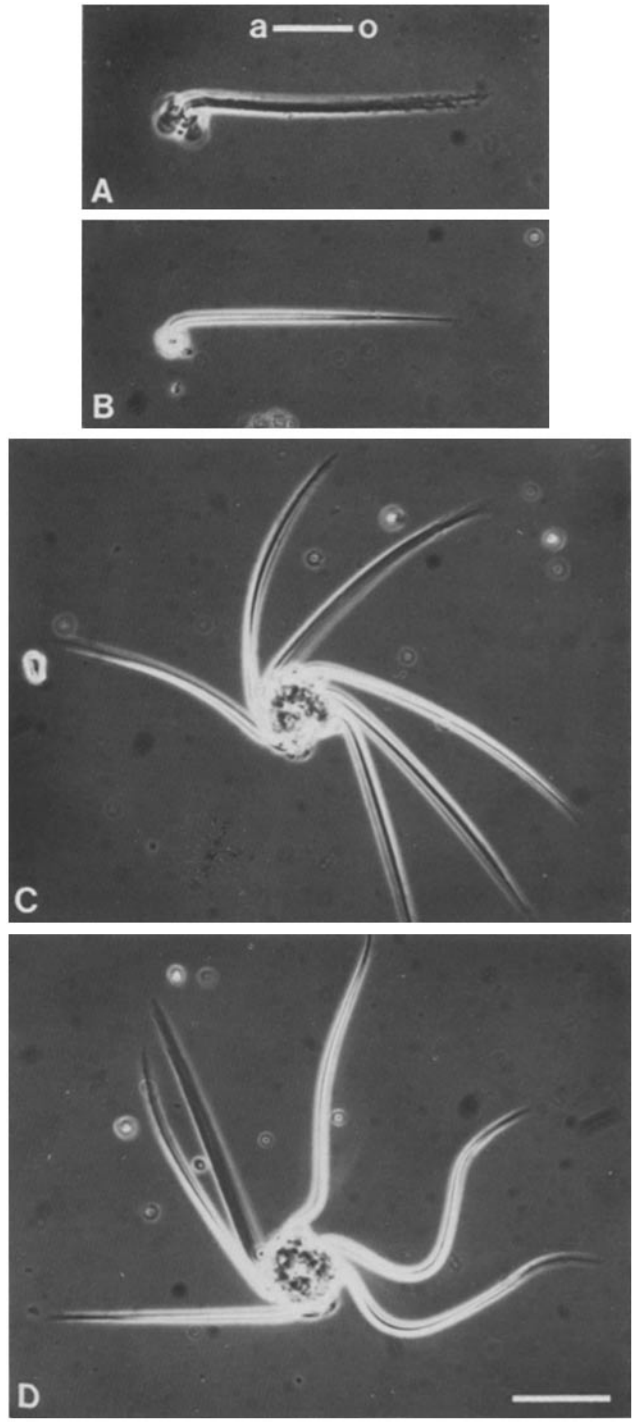

FIGURE 2 Comb plate cells isolated from living larvae by dissociation solution (*A*) or by cold treatment (*B–D*). A comb plate arises from one or more small epithelial cells and at rest retains an orally directed bend at the base (a—o, aboral–oral axis). (C and *D*) Six plates of an entire, dissociated comb row form a pinwheel which rotates when the plates beat (*D*). Electronic flash, phase-contrast optics. Bar, 50 μm. × 265.

of isolated comb plate cells and allowed us to determine whether their beat direction was normal or reversed.

In dissociation solution or CMF–ASW, comb plates of isolated cells beat in the normal direction with the effective stroke directed aborally (Fig. 3*A*). The form of the beat cycle resembled that of comb plates in intact larvae (see reference 29). Dissociated comb plate cells "swam" in circular paths

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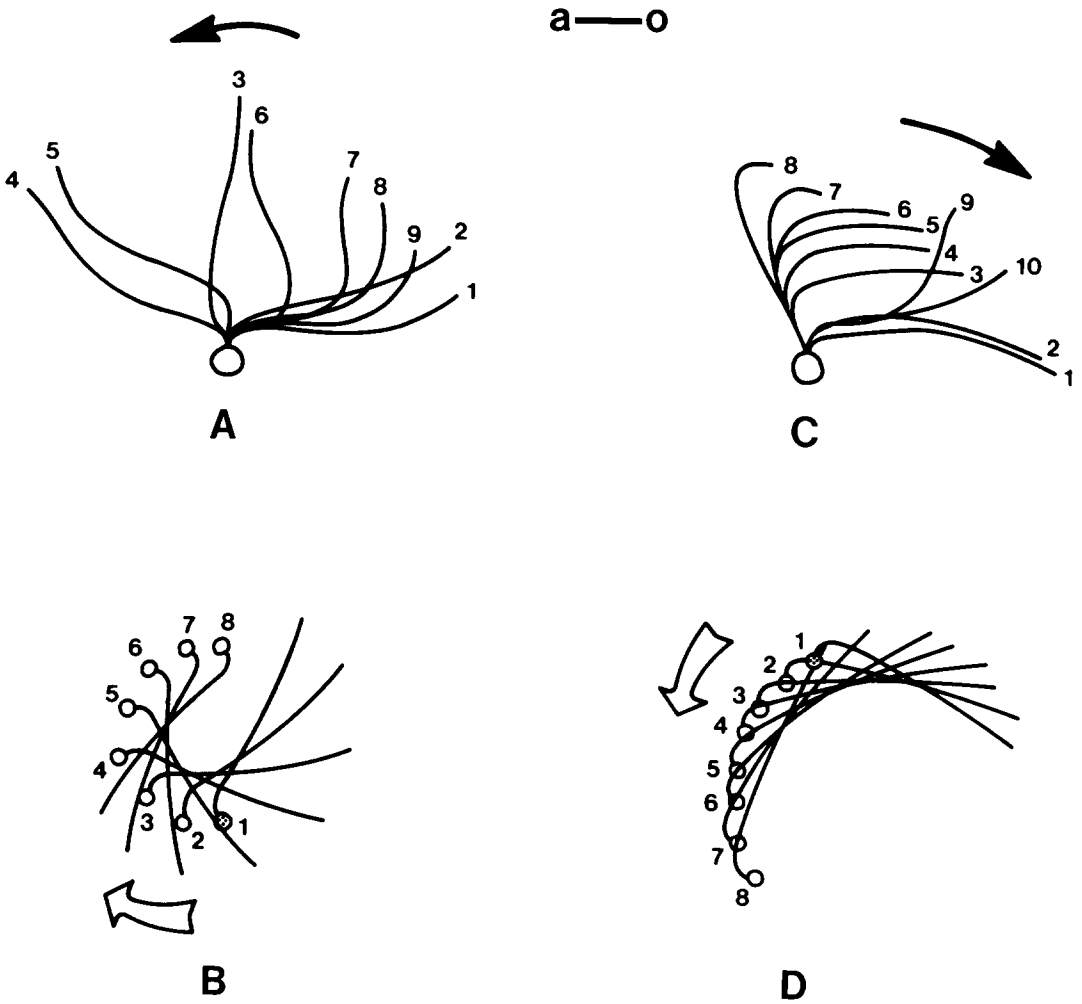

FIGURE 3 Tracings of video fields of beat form (*A* and *C*, at 1/60 s intervals) and swimming paths (*B* and *D*) of living dissociated comb plate cells. (*A*) In CMF-ASW the comb plate beats in the normal direction, starting with an effective stroke (arrow, fields 1-4) directed aborally (*a—o*, aboral-oral axis), followed by a recovery stroke (fields 5-9). (*B*) Part of the swimming path of a comb plate cell in CMF-ASW, showing successive rest positions at every third beat cycle. Note that the plate circles clockwise from position 1. (*C*) In CMF-ASW with 5 μM A23187 and 10 mM Ca, the comb plate beats in the reverse direction, beginning with a recovery stroke (fields 1-8) and followed by an effective stroke (arrow, fields 8-10) directed orally. (*D*) Part of the swimming path of an ionophore + Ca^{2+}-treated comb plate cell, showing successive rest positions at every fourth beat cycle. The plate circles counterclockwise from position 1.

with the aboral side foremost and the plate facing the inside of the circle (Fig. 3*B*). When the comb plate cell was oriented with its oral-aboral axis from right to left and the plate uppermost, it always circled in a clockwise direction (Fig. 3*B*).

In CMF-ASW containing 5 μM A23187 and 5 or 10 mM Ca^{2+}, dissociated comb plates often beat in the reversed direction. The reversed beat cycle began with an aborally directed recovery stroke and was followed by an effective stroke in the oral direction (Fig. 3*C*). Comb plate cells beating in reverse also followed circular paths with the aboral side leading, but the plate faced the outside of the circle and the cell moved counterclockwise (Fig. 3*D*). It is curious that this means that the direction of circling was opposite the direction of the recovery stroke during normal beating but opposite the direction of the effective stroke during reversed beating. We do not yet understand the reason for this difference. Reversed beating also occurred occasionally in CMF-ASW containing A23187 without added Ca^{2+}, but not if 1 mM EGTA was included; this indicates the presence of trace amounts of residual Ca^{2+}.

ATP-reactivated Models of Comb Plates

Permeabilized, reactivated models of comb plates allowed a more quantitative determination of the effects of Ca^{2+} on beat direction. Extracted larvae in WS appeared slightly shrunken and distorted (Fig. 4*A*) and were easily disrupted by mechanical forces such as compression by a coverslip. No beating of comb plates was usually observed in WS, although occasionally a few comb plates beat weakly for several minutes before stopping.

In the presence of ATP (RS), comb plates beat actively and continuously for up to several hours (Fig. 4, *B-E*). All the cilia within a single plate beat synchronously. The vigorous movements of reactivated plates usually detached their cells from the fragile larval body (Fig. 4, *D* and *E*), resulting in dissociated, reactivated comb plate cells much like living, dissociated cells described above.

In RS without Ca^{2+}, plates beat in the normal direction with the effective stroke directed aborally (Fig. 5*A*). The form of the beat cycle was similar to that of dissociated living cells (compare Fig. 3*A*). Beat frequency and extent of reactivation

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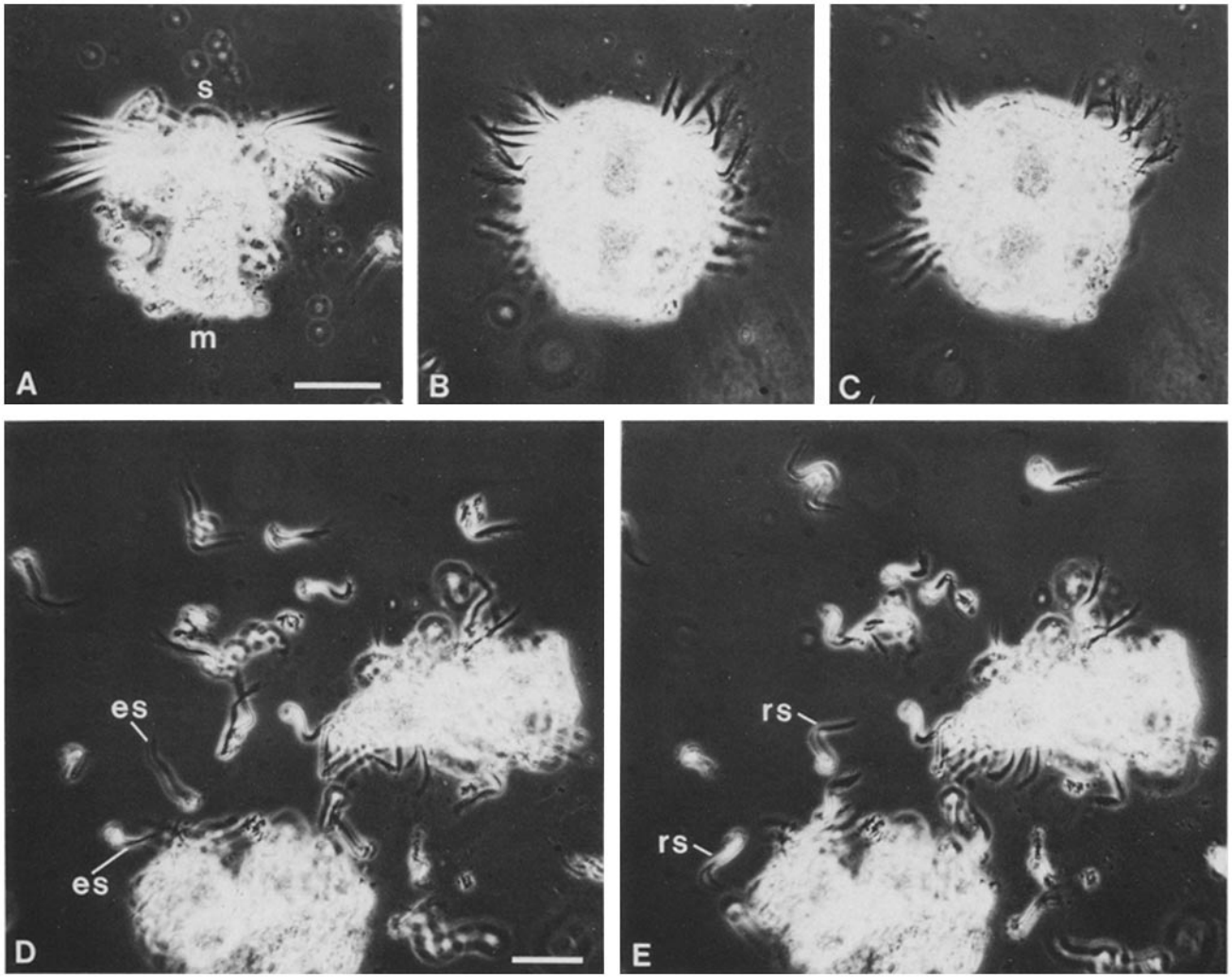

FIGURE 4 Permeabilized models of *Mnemiopsis* comb plates. (*A*) Extracted larva, in WS, which shows nonbeating comb plates projecting from either side (*s*, statocyst; *m*, mouth). (*B* and *C*) Reactivated larva in RS (with ATP), which shows beating plates in different positions at successive times. (*D* and *E*) Reactivated larvae in RS after many beating comb plate cells have detached from the body. In *D*, two isolated comb plate cells are performing effective strokes (*es*); in *E*, these plates are now in the recovery stroke (*rs*). Electronic flash, phase-contrast optics. (*A–C*) Bar, 100 μm. × 120. (*D* and *E*) Bar, 100 μm. × 105.

depended on the Mg^{2+}–ATP concentration, ranging from poor or no reactivated beating at 0.1 mM ATP to maximum frequency at 2.5 mM ATP. In standard RS containing 2 mM ATP, the average beat frequency was 6.2 ± 1.5 Hz (n = 48; Fig. 6). ADP (2–4 mM) supported only slow beating of a small number of comb plate cells.

Reactivated beating was inhibited by 2 mM *N*-ethyl maleimide, 1 mM mersalyl acid, or 1 mM *p*-hydroxymercuribenzoate. 1 μM sodium vanadate in RS inhibited or slowed the beating of many plates, 10 μM vanadate inhibited reactivation of all but a few, weakly moving plates, and 50 μM vanadate completely inhibited all reactivated motility. Addition of 2.5 mM norepinephrine reversed vandate inhibition of reactivation, resulting in resumption of beating by many comb plates within a few minutes.

The direction of reactivated beating was determined at various concentrations of free Ca^{2+} in RS. At concentrations $< 5 \times 10^{-7}$ M Ca^{2+}, plates beat exclusively in the normal direction (Table I and Fig. 5, *A* and *B*). At higher Ca^{2+} concentrations (up to 2×10^{-5} M), about two-thirds of the plates beat in the reversed direction, one-quarter of the plates beat in a pattern intermediate between normal and reversed forms (Fig. 5*C*), and the remaining plates beat in the normal direction (Table I). As in Ca^{2+}-ionophore–treated living comb plate cells (Fig. 3*C*), reversed beating of reactivated plates began with a distally propagated recovery bend which unrolled the plate in the aboral direction and was followed by a power stroke in the oral direction (Fig. 5*D*).

The frequency of reactivated beating was also determined as a function of free Ca^{2+} concentration (Fig. 6). Since plates showing Ca^{2+}-induced reversal might be more likely to exhibit possible Ca^{2+}-dependent frequency responses than those beating in the normal direction, we used only plates beating in the reversed direction to calculate beat frequencies at 10^{-6} and 10^{-5} M Ca^{2+} in Fig. 6. It can be seen that beat frequency did not change significantly at different Ca^{2+} concentrations in RS. In fact, at 10^{-6} and 10^{-5} M Ca^{2+} the frequencies of plates beating in normal vs. reversed directions were similar (data not shown).

Electron Microscopy of Comb Plate Models

The fine structure of comb plates from living adult and larval ctenophores has been described previously (1, 6, 29). In brief, the component cilia are linked into parallel rows by

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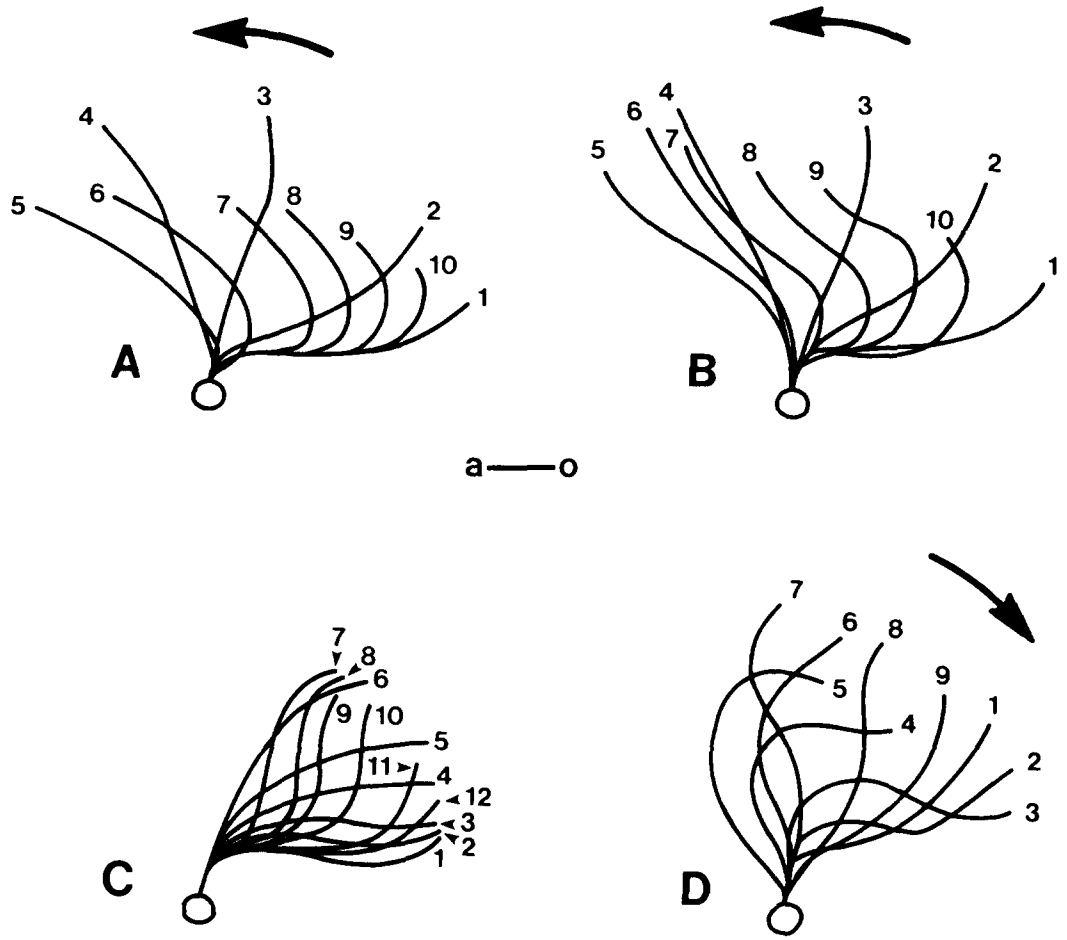

FIGURE 5 Tracings of successive video fields (1/60 s intervals) of beat cycles of reactivated comb plate cells at different concentrations of free Ca^{2+}. Arrows show direction of effective stroke. All plates are similarly oriented with respect to the aboral–oral axis (*a*—*o*). (*A*) No added Ca^{2+}. The comb plate beats in the normal direction with the effective stroke (fields 1–4) directed aborally and followed by a recovery stroke (fields 5–10). (*B*) 10^{-7} M Ca^{2+}. The effective stroke is still in the aboral direction. (*C*) 10^{-6} M Ca^{2+}. An example of a beat pattern intermediate between normal and reversed beating. (*D*) 10^{-6} M Ca^{2+}. The beat cycle is reversed, starting with a recovery stroke (fields 1–5) and followed by an effective stroke (fields 6–9) in the oral direction.

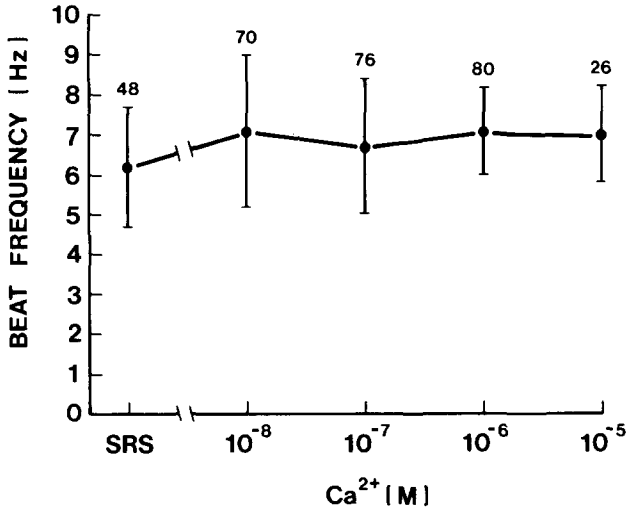

FIGURE 6 Beat frequency of reactivated comb plates as a function of free Ca^{2+} concentration in RS. Each data point is the mean ± SD for the number of different comb plates indicated. Each Ca^{2+} concentration represents a range of ± 0.5 pCa units. *SRS*, standard reactivation solution without added Ca^{2+}. Frequency measurements at 10^{-6} and 10^{-5} M Ca^{2+} were based exclusively on plates beating in the reverse direction (see text).

TABLE I

Beat Direction of Reactivated Comb Plates vs. Free Ca^{2+} Concentration

	Ca^{2+} Concentration (*M*)				
	SRS	10^{-8}	10^{-7}	10^{-6}	10^{-5}
Beat direction (%)					
Normal	100	100	100	5	12
Intermediate	0	0	0	25	24
Reversed	0	0	0	70	64
n	48	70	81	115	41

Each Ca^{2+} concentration includes a range of ±0.5 pCa units. SRS, standard reactivation solution without added Ca^{2+}. *n*, number of different comb plates at each Ca^{2+} concentration.

compartmenting lamellae that extend from doublet 3 of one axoneme to doublet 8 of the neighboring axoneme (Fig. 7, *inset*). These rows run perpendicular to the plane of bending.

In ATP-reactivated comb plates, the rows of cilia are disorganized (Fig. 7). Few if any demembranated axonemes are present; instead, apparently continuous membranes surround individual axonemes or groups of axonemes, the latter resulting from fusion of membranes of adjacent cilia. This evident integrity of the ciliary membranes conceals their permeability

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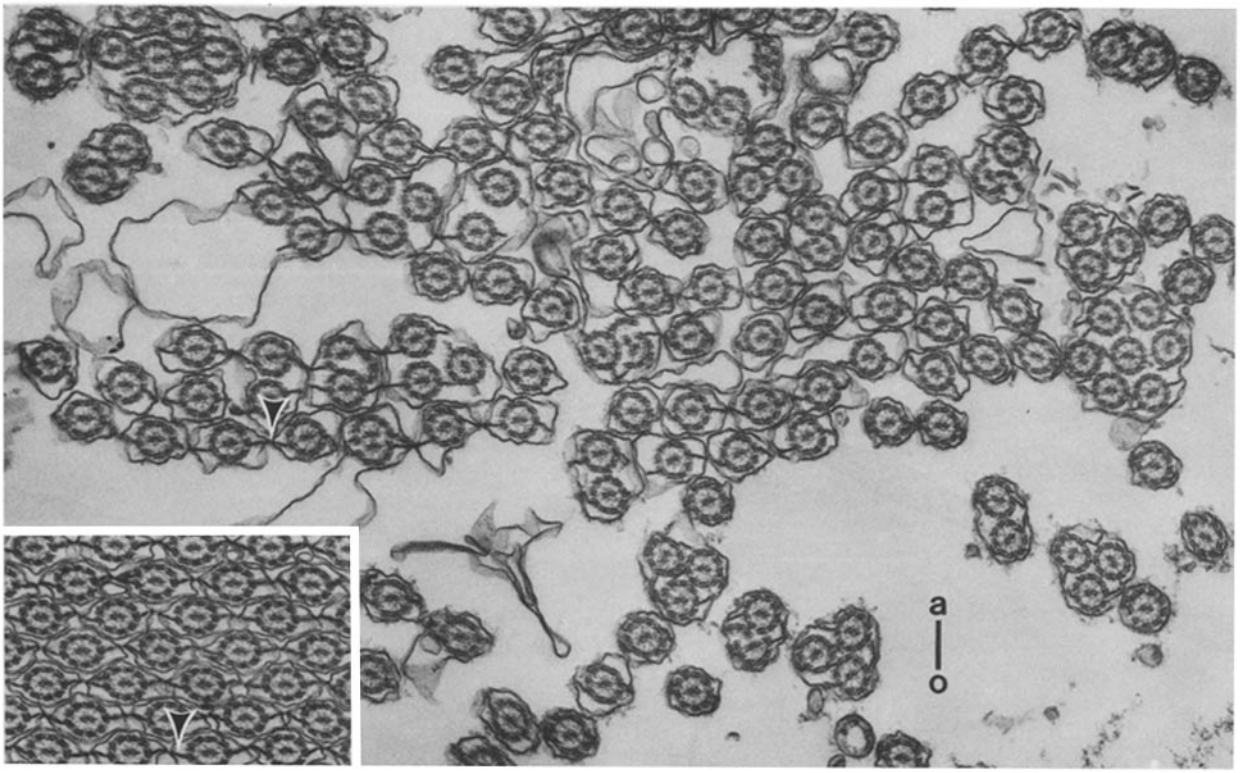

FIGURE 7 Electron micrographs of cross sections through comb plates from a reactivated larva and a living larva (*inset*). The rows of cilia in the reactivated comb plate are disorganized and membranes of adjacent cilia are often fused to surround groups of axonemes. Compartmenting lamellae (arrowheads) join doublets 3 and 8 of adjacent axonemes into rows running at right angles to the plane of bending. The normal direction of effective stroke is towards the top of the figure. *a*—*o*, aboral–oral axis. × 27,400.

to small molecules and ions. Compartmenting lamellae are retained and occasionally still link neighboring axonemes.

DISCUSSION

We previously showed that KCl induces ciliary reversal in larvae of the ctenophore *Pleurobrachia* and that this response is Ca^{2+} dependent (29). The reversal response of *Mnemiopsis* larvae, in contrast, is not triggered by an increase in the external KCl concentration but by an elevated Ca^{2+} concentration, even at normal KCl levels (29). Although this difference between *Pleurobrachia* and *Mnemiopsis* may reflect differences in possible nervous control of ciliary reversal in larvae of the two ctenophores, in neither case was it possible to distinguish whether Ca^{2+} was acting directly on the cilia themselves, and/or was required for synaptic triggering of the reversal response (29).

By treating isolated comb plate cells of *Mnemiopsis* larvae with Ca^{2+} ionophore and by determining the effects of Ca^{2+} on beat direction of permeabilized, ATP-reactivated comb plates, we have demonstrated here a direct involvement of Ca^{2+} in controlling ciliary beat direction in a metazoan system. Ciliary reversal in ctenophores thus resembles other known motor responses of cilia and flagella in being activated by an increase in intracellular free Ca^{2+} concentration. It remains to be shown whether ciliary reversal in larval ctenophores is under nervous control, as probably is true in adults (15, 28), or whether Ca^{2+} is solely required for activation of the reversal machinery itself.

Our development of an ATP-reactivated model of comb plates greatly facilitates investigation of the mechanism of Ca^{2+} control of ciliary reversal. To our knowledge, this is the first case of reactivation of a typical compound ciliary organelle; the only related case is our recent reactivation of macrocilia from the ctenophore *Beroë*, which are unique in having a single outer membrane surrounding the multiple axonemes (30).

We found that the several hundred cilia of a permeabilized, ATP-reactivated comb plate still beat synchronously. This confirms recent microsurgical experiments on living, adult comb plates, which show that synchronization of beating is due to hydrodynamic coupling between the component cilia (26).

The use of reactivated models allowed us to determine quantitatively the Ca^{2+} dependence of the reversal response. A free Ca^{2+} concentration > 10^{-6} M was required to obtain reversed beating of reactivated comb plates. This Ca^{2+} level is similar to that needed to induce various types of ciliary and flagellar motor responses in other reactivated systems (2, 4, 5, 10, 11, 31, 32). In particular, Triton-extracted models of *Paramecium* undergo a change in the direction of ciliary effective stroke and swim backwards when the Ca^{2+} concentration exceeds 10^{-6} M (17, 18).

In vivo, ciliary reversal in protozoa and larval or adult ctenophores is accompanied by an increase in beat frequency (12–14, 28, 29). It has been suggested (13, 14) that beat frequency in ciliates is regulated by intracellular Ca^{2+} in a bimodal fashion: frequency maxima occur at low (10^{-7} M) and high (>10^{-6} M) Ca^{2+} concentrations (corresponding to increased forward swimming and backward swimming, respectively), and a frequency minimum occurs at an intermediate Ca^{2+} concentration (corresponding to a transitional inactive state). Evidence against this view (see also reference 3)

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includes an early study of Triton-extracted models of *Paramecium*, which did not show any notable effect of Ca^{2+} on beat frequency (17). Similarly, we failed to find any significant change in beat frequency of reactivated comb plates with changes in Ca^{2+} concentration. Recently, however, using a high Mg^{2+}-EGTA method for preparing Triton-extracted models of *Paramecium*, Nakaoka et al. (18) showed a bimodal dependence of beat frequency on Ca^{2+} concentration, similar to that described above. Evidently the earlier conditions of extraction destroyed the Ca^{2+} sensitivity of beat frequency but not of beat direction. It is likely, therefore, that improved extraction conditions for comb plates may restore a Ca^{2+} dependence of beat frequency in this system as well.

In conclusion, we have demonstrated that ciliary reversal in ctenophore comb plates is directly controlled by Ca^{2+}. This finding allows the experimental advantages of comb plates to be used for future studies on the Ca^{2+} regulation of ciliary motion, particularly the site and mechanism of action of Ca^{2+} within the ciliary axoneme. For example, in preliminary work we found that elastase or trypsin treatment (see reference 22) of reactivated comb plates induces ATP sliding disintegration of the microtubules (27). We plan to use the unique ultrastructural markers for specific doublet microtubules in comb plate cilia (1, 29) to test the effects of Ca^{2+} on the pattern of microtubule sliding during ciliary reversal. This approach has already yielded important information on microtubule sliding patterns during the normal beat cycle of ctenophore macrocilia (30).

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Please address correspondence to S. L. Tamm.

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