

## MECHANICAL SYNCHRONIZATION OF CILIARY BEATING WITHIN COMB PLATES OF CTENOPHORES

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### SUMMARY

The mechanism which synchronizes the beating of the hundreds of thousands of long cilia making up a ctenophore comb plate was investigated by microsurgical experiments on single comb plates of *Mnemiopsis* and *Pleurobrachia*. Comb plates of lobate ctenophores (e.g. *Mnemiopsis*) are triggered to beat by ciliated grooves which run between the centres of adjacent plates. By creating gaps or introducing mechanical barriers between two parts of a plate, or by severing the cells at the base of a plate, it was shown that physical proximity of cilia, not tissue continuity, is required for synchronization of beating. In *Pleurobrachia* only the first comb plate of each row is activated by a ciliated groove, and similar experiments to those done on *Mnemiopsis* gave identical results. Although adjacent comb plates in *Pleurobrachia* are triggered mechanically by movements of the preceding plates without the need for an intraplate synchronizing mechanism, unilateral amputation of a plate showed that cilia within these plates may also be synchronized by mechanical coupling. Therefore, in cases where the beating of a comb plate is triggered by a ciliated groove – either at the head of a comb row (in all ctenophores) or along the row (lobates only) – the cilia within the plate are synchronized by hydrodynamic coupling forces between them, not by electrical coupling between their cells as assumed previously.

### INTRODUCTION

The mechanism of metachronal coordination of ciliary activity has long been studied, and has been shown in all cases to depend on hydrodynamic interaction (viscous-mechanical coupling) between neighbouring cilia (Machemer, 1974; Murakami, 1963; Sleight, 1974; Tamm, 1973, 1982, 1983). Little is known, however, about the mechanism which synchronizes the beating of component cilia within a compound ciliary organelle, so that all the cilia beat together as a single unit. Examples of compound cilia include the cirri and membranelles of protozoa, the large abfrontal cilium and laterofrontal cilium of mussel gills, macrocilia of the ctenophore *Beroë*, and the giant comb plates of ctenophores. Microsurgical experiments on certain compound cilia indicate that synchronization of beating is achieved by mechanical coupling between the constituent cilia (Carter, 1924; Chambers & Dawson, 1925; Tsuchiya, 1969). For example, when the abfrontal cilium or laterofrontal cilium of *Mytilus* is split into component cilia by a

microneedle, the cilia beat out of unison (Carter, 1924; Tsuchiya, 1969); upon removing the needle the individual cilia reunite and beat together as a single unit again. Alternatively, membrane excitation and cell-to-cell electrical coupling have been proposed to account for the synchronous beating of the hundreds of thousands of long cilia, borne on a ridge of thousands of cells, which make up a ctenophore comb plate (Horridge & Mackay, 1964; Satterlie & Case, 1978). To date, no experimental studies have been made on the mechanism of ciliary synchrony in ctenophores. In this report I have investigated this problem by performing microsurgical experiments on single comb plates. I show that the cilia within a comb plate are synchronized by mechanical coupling forces between them, not by electrical signals between their cells as assumed previously.

#### MATERIALS AND METHODS

*Mnemiopsis leidyi* and *Pleurobrachia pileus* were dipped carefully from the surface of Great Harbor or Vineyard Sound near Woods Hole, Mass. Ctenophores were maintained in excellent condition in perforated buckets submerged in running sea water, and were fed daily with freshly caught plankton.

Long pieces of comb rows were cut out of the animal and held stationary in a 'micro-vice' apparatus mounted in a Lucite chamber as described previously (Tamm, 1973). A mixture of sea water and 7.0%  $MgCl_2$  (1:1) was commonly used in place of sea water for *Mnemiopsis* to prevent muscular retraction of the comb row; initiation and transmission of metachronal ciliary waves are not affected by excess  $Mg^{2+}$  (Horridge, 1965; Tamm, 1982). The preparation was viewed under a dissecting microscope by dark-field or bright-field transmitted light.

Glass microneedles were made from capillary tubing drawn out in an electrode puller, or pulled by hand in a small flame. Needles and other micro-tools were operated by a Jena micromanipulator. Amputation of comb plates (Fig. 3B,C) was done with iridectomy scissors (Weiss, London).

Ciné films of certain experiments were taken through the dissecting microscope with a 16 mm ciné camera at 25 or 50 frames  $s^{-1}$ . Higher resolution images of microsurgical results were obtained by transferring the preparation to a microscope slide and viewing with Zeiss Nomarski optics using a 16 $\times$  or 40 $\times$  objective. Photographs (Fig. 2) were taken on Kodak High Contrast Copy film.

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Fig. 1. Microsurgical experiments on intraplate coordination using comb plates of *Mnemiopsis*. Metachronal waves along the comb rows and ciliated grooves (*cg*) travel from reader's right to left, in an aboral-oral direction (A—O). The effective stroke of all cilia is to the right (arrows). (A) Separation produced between two parts of a single plate by holding back an intermediate piece with a needle. Only the larger part joined to the ciliated groove is triggered to beat (arrow) by the ciliated groove during passage of a metachronal wave down the row. The ciliated groove widens at its junction with the aboral side of each comb plate. The plate to the left is held down with a glass rod to prevent its mechanical stimulation of the immobile part of the experimental plate in the reverse direction. (B) Similar experiment to A except that the middle part of a plate is restrained with a needle. Beating of the ciliated groove triggers twitches of this piece (arrow), but the two separated end parts do not beat. (C) Effect of slitting a plate longitudinally with a razor blade without cutting the underlying tissue. Metachronal waves trigger beating of the larger part of the plate connected to the ciliated groove (arrow), but not the end piece. (D),(E) Effect of cutting through the ridge of comb plate cells, as well as producing a narrow cleft in the plate itself. The two severed parts beat together (arrows, D); however, if the end piece is temporarily moved away from the main part by pushing against its base with a needle, it no longer beats with the main part (E).

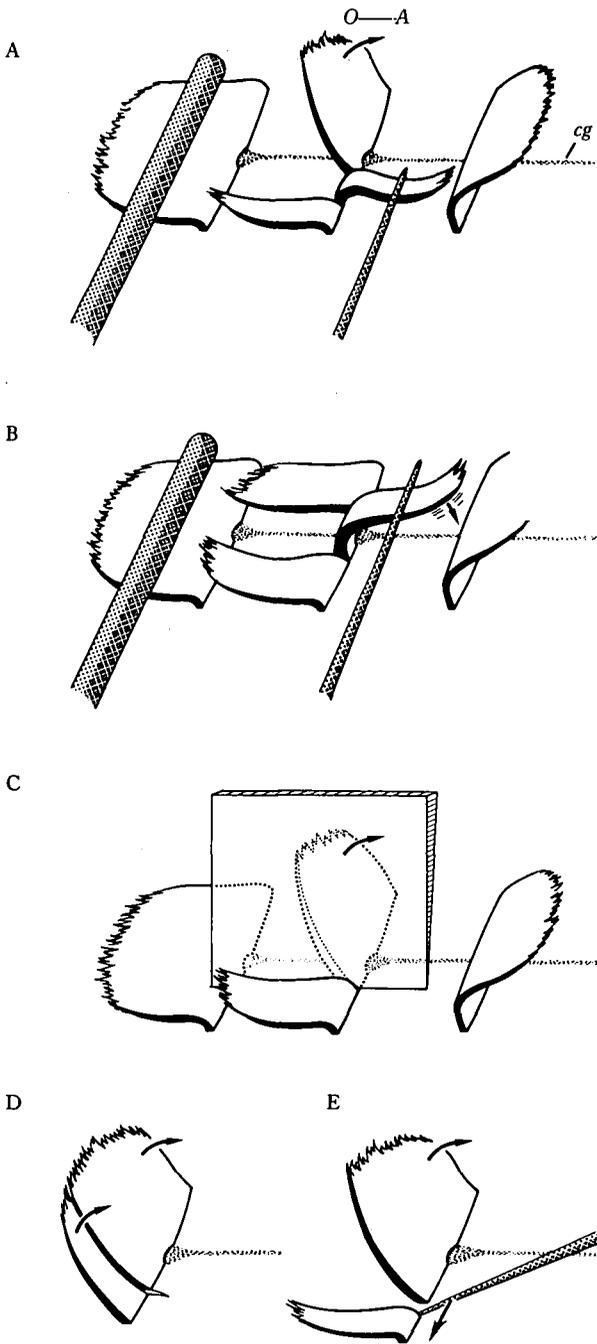


Fig. 1

## RESULTS

In all ctenophores, the first comb plate at the head of each comb row is triggered to beat by a narrow tract of short cilia, the ciliated groove, which runs from the aboral statocyst to the centre of the base of the plate (Tamm, 1973, 1982). In lobate ctenophores (e.g. *Mnemiopsis*) the ciliated grooves continue from plate to plate along the comb rows, and trigger the beat of adjacent plates during metachronal coordination (Tamm, 1973, 1982). A signal must therefore be transmitted outward from the ciliated groove junction to either side of a comb plate to activate beating of all the cilia within the plate.

To investigate the nature of this synchronal coordination, microsurgical experiments were performed on single comb plates along comb rows of *Mnemiopsis* (Fig. 1). Ciné films of some of these experiments have been presented previously (Tamm, 1980). First, a physical gap between two parts of a plate was made by slitting a plate longitudinally into three components with a microneedle, and bending back the middle part close to the body surface (Fig. 1A,B). If one of the side pieces was wider and connected to the ciliated groove, only this part beat during passage of a metachronal wave down the row; the other free piece, separated by a gap, did not beat (Fig. 1A). This effect was reversible: upon releasing the intermediate sliver the parts reunited and the whole plate beat as a unit again. At high beat frequencies, however, the separated parts of the plate usually beat together regardless of the presence of a gap between them. If the restrained part of the plate was medial and connected to the ciliated groove, metachronal waves caused twitches of this part, but no movements of the two free end pieces (Fig. 1B). Release of the middle part resulted in synchronous beating of the entire plate.

A mechanical discontinuity between two parts of a plate, without preventing the movement of an intermediate piece, was made by slitting a plate (but not the underlying tissue) with a chip of razor blade held just above the tissue surface (Fig. 1C). Only the larger part of the plate connected to the ciliated groove was stimulated to beat by metachronal waves; the narrower part on the other side of the barrier did not beat. Upon removing the razor blade, both parts reunited and beat synchronously again.

Lastly, a deep cut was made with a glass needle across the ridge of polster cells at the base of a comb plate, producing a narrow cleft in the plate as well (Figs 1D,E, 2). Nevertheless, the two severed parts of the plate beat together. However, if the smaller part was moved a short distance away from the main part with a needle, this piece no longer beat with the main part of the plate (Fig. 1E). Upon pushing the end part back against the main part to close the gap, the entire plate resumed synchronal beating. Physical proximity, not tissue continuity, is thus required for synchronization of beating between parts of a comb plate.

Similar microsurgical experiments on the first comb plate of a row in *Pleurobrachia* gave identical results.

In *Pleurobrachia*, other cydippids and beroid ctenophores the ciliated groove ends at the first comb plate and does not continue down the row. In these species adjacent comb plates are triggered to beat by hydrodynamic coupling forces arising from the movements of the plates themselves (Tamm, 1973, 1982). Each cilium within a plate may thus be stimulated independently by water movements generated by the active

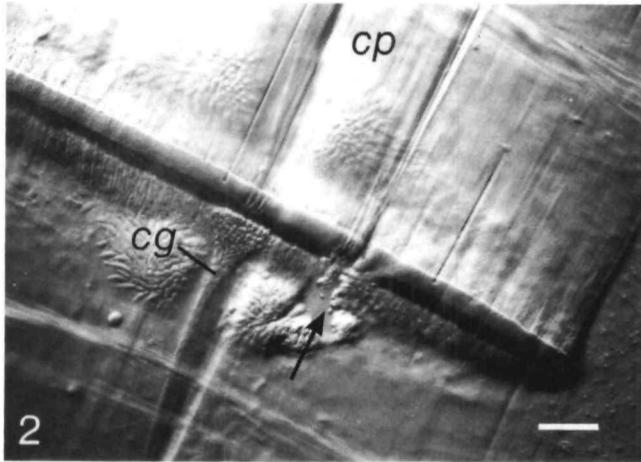


Fig. 2. Comb plate (*cp*) of *Mnemiopsis* after cutting through its base with a glass needle, as shown in Fig. 1D,E. The ridge of polster cells is severed as well as the plate itself (arrow). Note the widening of the ciliated groove (*cg*) at its junction with the aboral side of the plate. Zeiss Nomarski optics, electronic flash. Scale bar, 50  $\mu$ m.



stroke of the preceding plate, without the need for an intraplate synchronizing mechanism. That this is the case was shown by slitting a plate along a comb row of *Pleurobrachia* into three parts, and then pressing down the middle piece against the

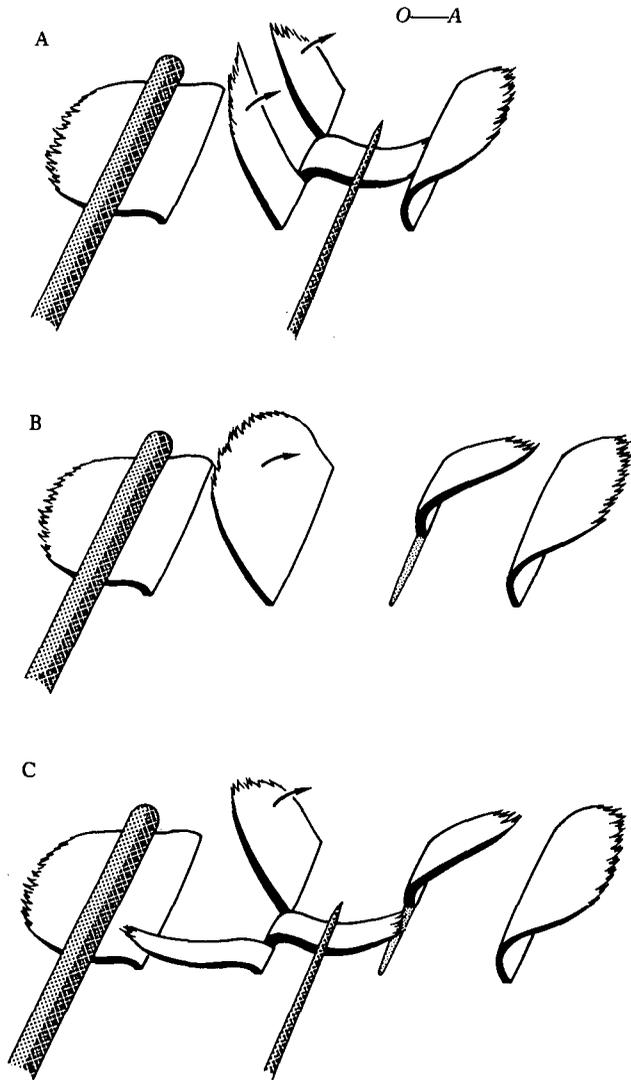


Fig. 3. Microsurgical experiments on single comb plates along a comb row of *Pleurobrachia*. Metachronal waves travel from reader's right to left (A—O, aboral—oral direction) with the effective stroke towards the right (arrows). (A) Separation produced between two parts of a plate by holding back the middle part with a needle. Beating of the plate to the right triggers synchronal beating of both end pieces (arrows), showing direct mechanical activation by the preceding plate. The plate to the left is immobilized to prevent reverse mechanical stimulation. (B) One side of the preceding plate is removed to prevent direct mechanical stimulation of the same side of the following plate. Nevertheless, this entire plate beats as a unit (arrow), demonstrating the existence of synchronous coordination within the plate. (C) Separation produced between the stimulated and unstimulated sides of a plate by holding back an intermediate piece with a needle. The separated end part does not beat when the main part is triggered to do so by the preceding half-plate (arrow), indicating that synchronous coordination is due to mechanical coupling between cilia within the plate.

body surface to create a gap between the two end parts (Fig. 3A). In contrast to the results with *Mnemiopsis* (Fig. 1B), both of the separated parts of the *Pleurobrachia* plate beat together during passage of metachronal waves down the row. Each end piece was thus stimulated directly by the movement of the preceding plate.

To test whether intraplate coordination can occur in *Pleurobrachia*, direct mechanical stimulation of one side of a plate by the preceding plate was avoided by amputating the corresponding side of the neighbouring plate (Fig. 3B). Nevertheless, the entire plate beat as a unit, demonstrating the existence of synchronal coordination between the side which was triggered by the active stroke of the neighbouring half-plate, and the other unstimulated side. To determine the nature of this coordination, a physical gap was made between the stimulated side and the unstimulated part of this plate by holding back an intermediate sliver with a needle. Activity of the neighbouring half-plate triggered beating of the corresponding side of the experimental plate, but not of its separated end piece (Fig. 3C). Upon release of the intermediate piece, the entire plate beat together when stimulated by the preceding half-plate.

#### DISCUSSION

The present results show that in cases where the beating of a plate is triggered by a ciliated groove – either at the head of a comb row (in all ctenophores) or along the row (in lobates) – the cilia within the plate are synchronized by hydrodynamic coupling forces between them. Such mechanical interaction is sufficient to ensure ciliary synchrony in naturally frayed plates, where the separation between adjacent parts is very narrow. As shown above, mechanical coupling is even possible across wider gaps if the cilia beat at high frequencies and create stronger water currents.

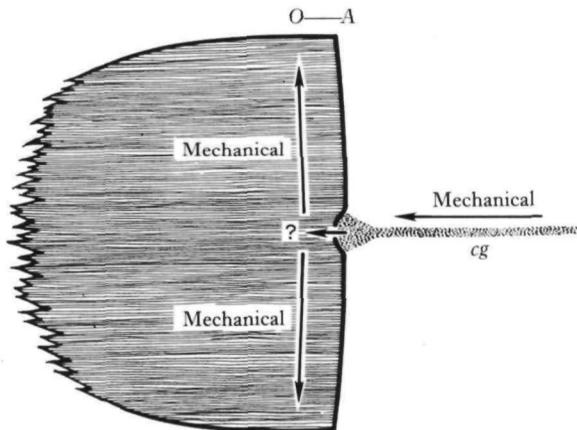


Fig. 4. Diagram of steps leading to synchronous activation of cilia within a comb plate by the ciliated groove, based on experiments shown in Fig. 1. Metachronal waves in the ciliated groove (*cg*, arrow) are coordinated by mechanical interaction (Tamm, 1982). At its junction on the aboral side of a comb plate, the ciliated groove excites beating of the central part of the plate by an unknown mechanism (arrow, question mark). Activation of beating spreads instantaneously to both sides of the plate by mechanical coupling between the cilia (lateral arrows), resulting in synchronal activity of the entire plate (A—O, aboral—oral direction).

The microsurgical experiments also show that electrical coupling between comb plate cells, suggested by the presence of gap junctions (Satterlie & Case, 1978), is not responsible for synchronizing the beating of cilia within a plate as proposed previously (Horridge & Mackay, 1964; Satterlie & Case, 1978). Gap junctions may serve to spread neurally-mediated signals for various motor responses of comb plates, such as ciliary arrest and reversal (Tamm, 1982; Tamm & Moss, 1985). This possibility is currently being investigated by intracellular electrophysiological recording and dye injection (A. G. Moss & S. L. Tamm, in preparation).

How the short cilia (10–15  $\mu\text{m}$ ) of the ciliated groove excite beating of the long cilia (>1000  $\mu\text{m}$ ) in the centre of a comb plate is not known. Since the cilia along the groove are coordinated by mechanical interaction (Tamm, 1982), they may stimulate the plate cilia directly ahead of them by a similar mechanism, providing this region of the plate is sufficiently mechanosensitive. The pronounced widening of the ciliated groove at its junction with the base of a comb plate (Fig. 2) suggests a device for mechanical amplification.

Once triggered by the ciliated groove, the beat is instantaneously transmitted outward to both sides of the comb plate by the high degree of mechanical coupling between neighbouring cilia (Fig. 4). The flange-like compartmenting lamellae which extend between adjacent cilia in the line of synchrony (Afzelius, 1961) undoubtedly contribute to this mechanical interaction.

The results on *Pleurobrachia* comb rows (Fig. 3) show that under conditions where intraplate coordination occurs, the cilia within the comb plates of cydippid ctenophores are also synchronized by mechanical coupling. In this regard, compartmenting lamellae are present between adjacent cilia in the comb plates of all ctenophores, regardless of the mechanism of metachronal coordination (Tamm, 1982). Although an intraplate synchronizing mechanism is normally not needed in *Pleurobrachia*, it may be advantageous in ensuring metachronal wave transmission in cases where comb plates have become partially damaged.

In conclusion, these findings emphasize the mechanosensitive nature of motile cilia (see Tamm, 1982, 1983; Wiederhold, 1976), and show that mechanical activation of the motile elements (Machin, 1958; Brokaw, 1966) plays a key role in the initiation and phasing of ciliary beating during synchronal as well as metachronal coordination.

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