THE ROLE OF CORTICAL ORIENTATION IN THE CONTROL OF THE DIRECTION OF CILIARY BEAT IN *PARAMECIUM*

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ABSTRACT

The swimming behavior of many ciliate protozoans depends on graded changes in the direction of the ciliary effective stroke in response to dèpolarizing stimuli (i.e., the avoiding reaction of *Paramecium*). We investigated the problem of whether the directional response of cilia with a variable plane of beat is related to the polarity of the cell as a whole or to the orientation of the cortical structures themselves. To do this, we used a stock of *Paramecium aurelia* with part of the cortex reversed 180°. We determined the relation of the orientation of the kineties (ciliary rows) to the direction of beat in these mosaic paramecia by cinemicrography of particle movements near living cells and by scanning electron microscopy of instantaneously fixed material. We found that the cilia of the inverted rows always beat in the direction opposite to that of normally oriented cilia during both forward and backward swimming. In addition, metachronal waves of ciliary coordination were present on the inverted patch, travelling in the direction opposite to those on the normal cortex. The reference point for the directional response of *Paramecium* cilia to stimuli thus resides within the cilia or their immediate cortical surroundings.

In metazoan cilia the direction of the effective stroke is usually restricted to one fixed plane. The cilia of many protozoans, on the other hand, are more versatile: the direction of their effective stroke may occur in almost any plane perpendicular to the body surface, resulting in changes in the direction of locomotion.

Electrophysiological studies on protozoans have shown that the direction of ciliary beating changes in response to depolarizing stimuli, and is continuously graded with membrane potential over large angles (Kinosita, 1954; Kinosita and Murakami, 1967). More recent work on *Paramecium* has pinpointed a voltage-dependent influx of Ca^{++} as the ionic signal controlling direction of beat (Eckert, 1972; Eckert and Naitoh, 1972; Naitoh and Kaneko, 1972, 1973).

Less is known about the structural basis of directional control. Tamm and Horridge (1970) demonstrated a morphological correlation between the orientation of the central pair of fibrils and the plane of ciliary bending during K^+-stimulated changes in direction of effective stroke in *Opalina* cilia. It remains to be shown, however, whether the orientation of the central pair determines the direction of beat or vice versa.

Regardless of the ionic and structural properties of the control mechanism, it seems clear that cilia with a variable plane of beat must have a frame of reference against which their directional response

to stimuli is measured. In this paper we have attempted to localize this reference point in cilia of *Paramecium*, by determining whether the functional orientation of the cilia is related to the polarity of the cell (organism) as a whole or to the orientation of the cortical structures themselves.

MATERIALS AND METHODS

Organisms and Culture Methods

Two stocks of syngen 4 of *Paramecium aurelia* were employed. The one used as a control, i.e. with all kineties normally oriented, was a kappa-free branch of the standard stock 51 (Sonneborn collection, see Sonneborn, 1970 *b*). The experimental stock, 3B1, consisted of mosaic cells carrying a patch of inverted kineties. 3B1 was derived from stock 51 as follows. On June 22, 1972, Dr. Mary L. Austin gave to one of the authors (T. M. Sonneborn) a pair of conjugants (see below for genotypes) which had remained united by a cytoplasmic bridge after conjugation was completed. Next day, of the seven cells that had been produced, two were normal singlets, four were homopolar doublets, and one—3B1, the progenitor of the experimental stock—was a heteropolar V-shaped cell. 3B1 gave rise during the following days to a considerable variety of cell lines differing in, among other things, their swimming behavior. Those which swam in circles (see below) were selected to be progenitors of the lines of descent used in the present study. During the course of the study, circlers were repeatedly selected to continue the culture which still, 21 mo later (March, 1974), is characterized by circling after several hundred cell generations. Selection was employed as needed to maintain the character because occasionally cells with less extremely aberrant swimming were produced, and these than failed to produce circling progeny.

The original conjugant pair was a cross between wild type stock 51 and a multiply marked clone homozygous for genes Pa (paranoiac, Kung, 1971 *a*, 1971 *b*), mtA (restriction to mating type VII, Byrne, 1973), tslll (temperature sensitive, dying at 36°C, Beisson and Rossignol, 1969), and A^{29} (a ciliary antigen gene derived from natural stock 29, Sonneborn, 1950). The resulting multiple heterozygote became homozygous at the first autogamy for Pa, mtA, tslll and A^{29}. The gene Pa confers hypersensitivity to Na^+ and results in spontaneous long backward swimming in Na^+-rich solutions.

Near the end of the present study, a stock of 3B1 wild type for Pa was derived to test for possible differential effects of Pa on ciliary activity in normal vs. inverted cortex. This stock was obtained (Schneller, personal communication) by crossing 3B1 with a stock 51 clone homozygous for tslll, A^{29}, and Pa^+. After autogamy, clones of 3B1 wild type for Pa were established by selecting F_2 cells that did not show prolonged ciliary reversals in Na^+-rich culture medium. The stock of 3B1 which is wild type for Pa will be denoted 3B1 Pa^+; that carrying Pa is designated simply 3B1.

The experimental and control stocks were grown in baked lettuce medium inoculated with *Aerobacter aerogenes* prepared as described by Sonneborn (1970 *b*, p. 251, section 4). The control stock 51 was reisolated weekly from an autogamous cell and grown at 27°C in tube cultures fed enough to permit one to two cell generations daily. From June 1972 to February 1973, the experimental stock 3B1 was grown at 25–27°C in depression slides and petri dishes with excess food; after February 1973, it was grown by M. V. Schneller at 13°C in depression slides with weekly reisolations, always with excess food, but was brought to room temperature before use. Because of its circling swimming, 3B1 was unable to survive in tube cultures: the cells, unable to swim up to the air-water interface, required shallow cultures in order to obtain oxygen.

Silver Staining

A modification of the silver nitrate impregnation technique of Chatton and Lwoff (1930) was used to identify the location and size of the inverted kinety region in 3B1 cells. Silver-stained preparations of 3B1 cells were made several times during the course of this study. In some cases populations of cells were split into two samples, one being used for silver preparations while the other was prepared for scanning electron microscopy.

Test Solutions

Unstimulated, forward swimming patterns were obtained in culture medium or in Dryl's (1959) solution (2 mM $Na_3C_6H_5O_7$, 1.5 mM $CaCl_2$, 2 mM Na_2HPO_4-NaH_2PO_4, pH 7.2).

Changes in the direction of ciliary beat were induced in 3B1 cells by the following treatments: (*a*) Ba^{++} solution (2 mM $BaCl_2$, 1 mM $CaCl_2$, 1 mM Tris, pH 7.0), which causes a long-lasting series of alternating reversals and normal forward movements (i.e., periodic ciliary reversal) in wild type cells (Dryl, 1961) and paranoiac mutants (Satow and Kung, 1974); (*b*) K^+ solution (25 mM KCl, in baked lettuce medium), which causes short-lasting continuous ciliary reversal in wild type paramecia (Jennings, 1906; Mast and Nadler, 1926) and paranoiac cells (Kung, 1971 *a*).

Swimming Path Records

A dark-field time-exposure method (Dryl, 1958) was used to photograph the swimming paths of paramecia under different conditions. Cells were placed in a thin layer of the test solution in a flat-bottomed depression slide. Dark-field photographs (1-s exposure) were taken through a Wild dissecting microscope on Kodak high-contrast copy 35-mm film (Eastman Kodak Co., Rochester, N. Y.).

Cinemicrography

The direction of ciliary beat in living paramecia was recorded by filming the movements of carmine particles near the cells. Films were made using bright-field optics (Zeiss Universal microscope with a × 16 planachromat objective, Carl Zeiss, Inc., N. Y.) and a Locam 16-mm camera (RedLake Labs, Santa Clara, Calif.) at 50 frames/s. Prints of selected frames were made from negatives of the original Kodak Tri-X reversal films (Eastman Kodak).

Scanning Electron Microscopy

Scanning electron microscopy was used to visualize directly the pattern of ciliary beating. Swimming paramecia were fixed instantaneously with osmium tetroxide (Parducz, 1967; Tamm, 1972) and dried by the critical point method (Anderson, 1951; Horridge and Tamm, 1969). Direct microscope observations of the swimming patterns at the time of fixation ensured that the cells were behaving as noted. Dried specimens were mounted on aluminum stubs, coated with gold-palladium, and viewed with an ETEC scanning electron microscope operating at 20 kV. Micrographs were taken on Polaroid Type 55 P/N film (Polaroid Corp., Cambridge, Mass.).

RESULTS

Cortical Structure

The kineties (ciliary rows) of *Paramecium* run generally in an antero-posterior direction (Figs. 1–3). A single kinety is composed of a row of cortical units. The structure and arrangement of cortical units in normally oriented kineties (NK) and inverted kineties (IK) are shown diagrammatically in Fig. 1.

The cortical geography of inverted kineties has been described in detail by Beisson and Sonneborn (1965) and Sonneborn (1970 *a*). The antero-posterior and right-left polarities of inverted kineties are reversed, "equivalent to 180° rotation in the plane of the body surface" (Beisson and Sonneborn, 1965). The inverted kinety stock used in this paper (3B1) had a greater number of inverted kineties than previously studied IK stocks: a patch of 15–20 adjacent inverted rows extended from about the 15th kinety to the left of the vestibule around to the middorsal region (Fig. 3).

Swimming Behavior

Wild type paramecia swim forward in a straight direction with a left-hand spiral path (Fig. 4 *a*). The 3B1 paramecia used in this study move in circles, anterior end foremost, with little or no net forward progression (Fig. 4 *b*). Two patterns of

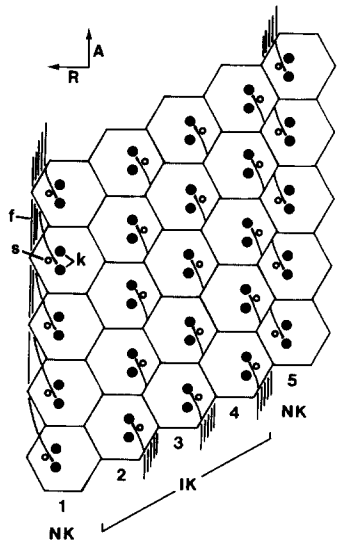


FIGURE 1 Diagram of the structure and arrangement of cortical units on the surface of *P. aurelia* with inverted kineties. Part of two normally oriented rows of cortical units (*NK*, 1 and 5) are depicted flanking three adjacent rows that are inverted (*IK*, 2–4). Our actual IK stock (3B1) carried 15–20 adjacent inverted rows. In describing the asymmetry of cortical units, "to the right" (*R* arrow) means in the clockwise direction around the cell when facing its anterior end (*A* arrow); "to the left" refers to the anticlockwise direction. A normally oriented cortical unit (rows 1 and 5) includes one or two kinetosomes (*k*) lying slightly to the right of the unit midline (only two-kinetosome units are shown here; units with one kinetosome lack the anterior one), a parasomal sac (*s*) to the right of the kinetosomes, and a kinetodesmal fiber (*f*) emerging to the right and extending anteriorly from the posterior kinetosome (when two are present). In silver preparations of normally oriented kineties, the kinetosome and parasomal sac appear as a short dash pointing obliquely to the animal's right, in one-kinetosome units, and as a triangle of three dots, also pointing to the right, in units with two kinetosomes (cf. Figs. 2 and 3). Inverted cortical units contain the same structures as normally oriented ones, but with reversed polarity (rows 2–4): the kinetosomes and parasomal sac lie to the left of the unit midline and the kinetodesmal fibers emerge to the left and run posteriorly. In silver preparations of inverted kineties, the tripartite triangular granules representing two-kinetosome units, therefore, point to the left (cf. Fig. 3). A group of inverted kineties is bounded on its right side by an unusually wide space between oppositely pointing units in adjoining inverted and normal rows (1 and 2, see Fig. 3 *a*), and, on its left side, by an unusually narrow space where the visible structures point towards each other and are closer together (rows 4 and 5; see Fig. 3 *b*). Kinetodesmal fibers are not stained by silver impregnation, but their opposite

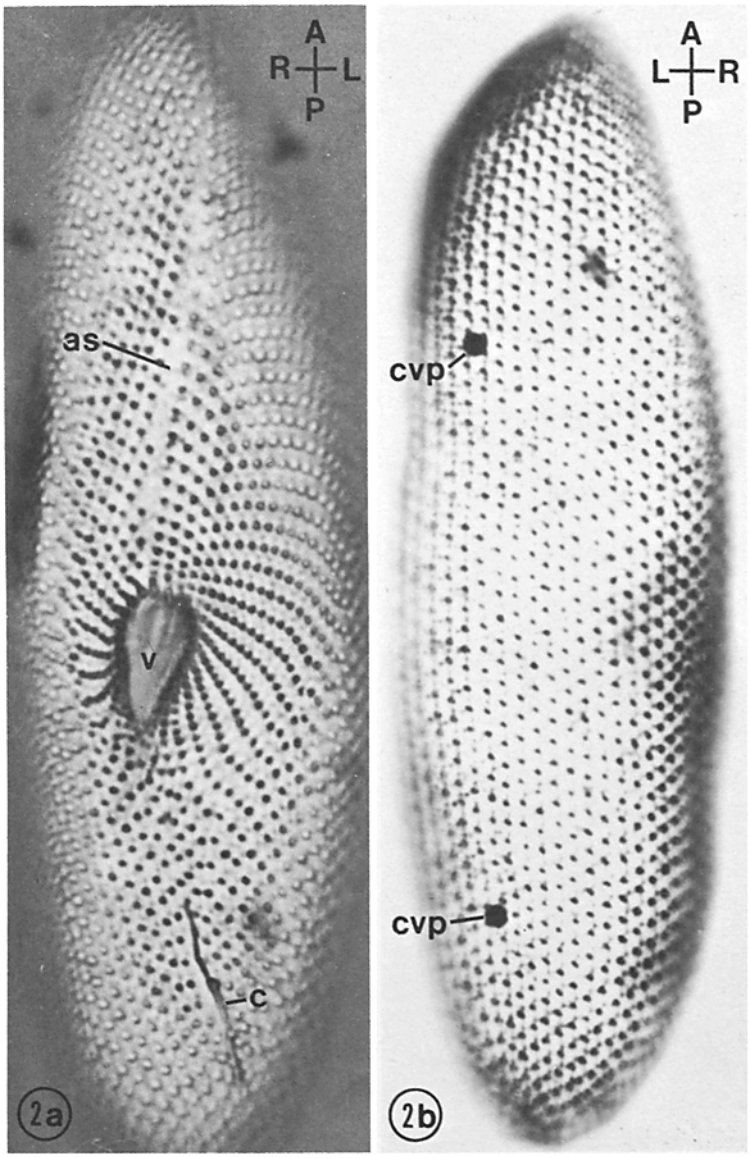


FIGURE 2 Cortical geography of wild type *P. aurelia* (stock 51). Ventral (*a*) and dorsal (*b*) surfaces of silver nitrate-impregnated cells (as viewed from outside the cell), showing the characteristic paths of the kineties. The one or two kinetosomes and parasomal sac of each cortical unit lie close together and appear as a single granule (silver deposit) in this figure, but they are partially resolvable in Fig. 3. *as*, anterior suture; *c*, cytoproct; *cvp*, contractile vacuole pore; *v*, vestibule, leading to gullet; *A*, anterior; *P*, posterior, *R*, right; *L*, left. × 1,250.

backward swimming, correlated with different stimulus conditions, were observed in these 3B1 cells. In normal culture medium or in Ba^{++}-carmine suspension they sometimes swam backwards in circles (Fig. 7). However, in K^+solution (or in Na^+-rich solution or Na^+-rich culture medium) the cells commonly swam backwards in a tight spiral path with a straight axis (backward, straight swimming, Fig. 4 *c*).

Although it was clear in earlier work (Beisson and Sonneborn, 1965) that the possession of

polarity in inverted rows has been demonstrated in digitonin preparations (Beisson and Sonneborn, 1965).

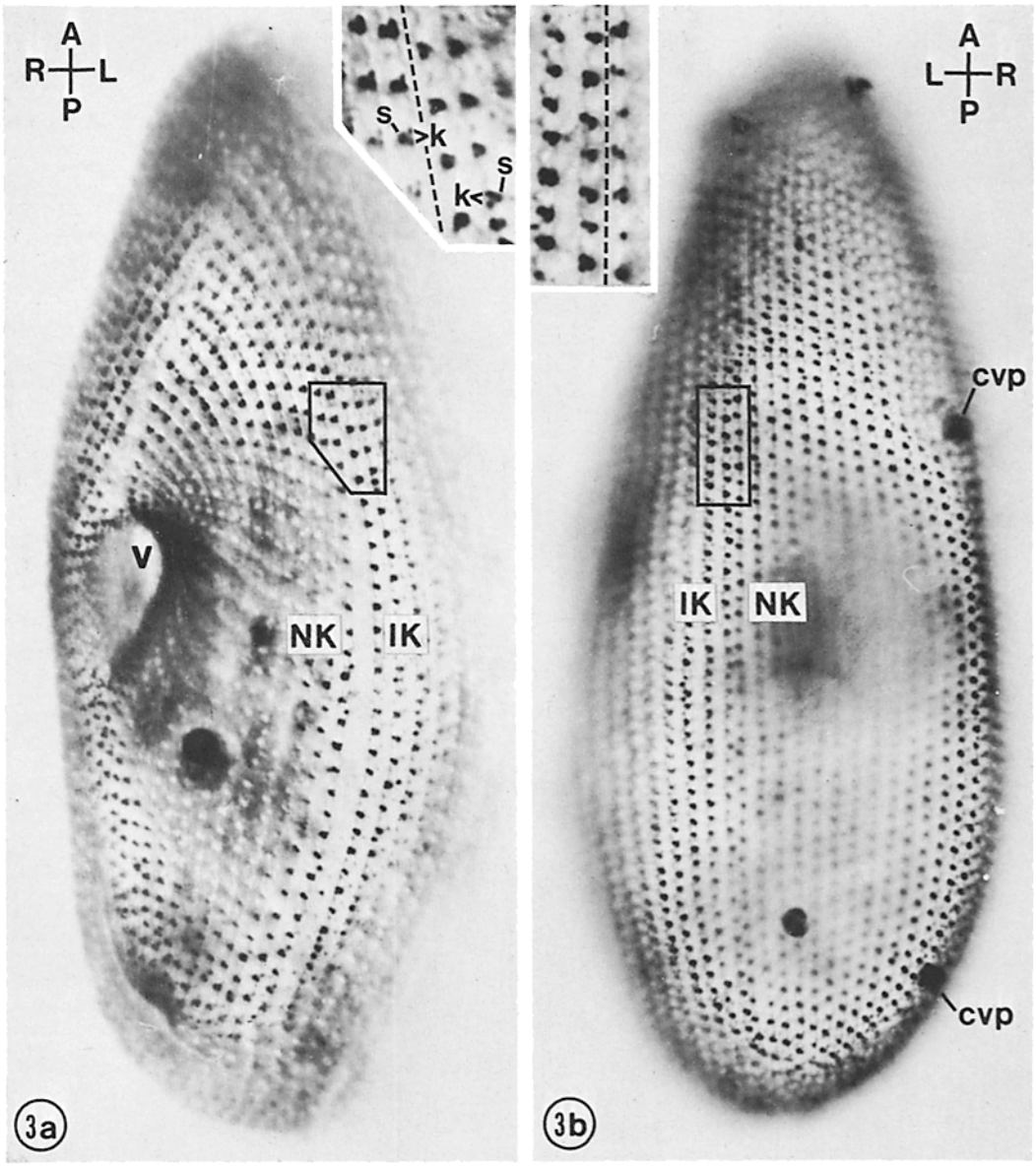


FIGURE 3 Cortical geography of 3B1 *P. aurelia* with inverted kineties. Left ventral (*a*) and left dorsal (*b*) surfaces of silver nitrate-impregnated cells (as viewed from the outside). Because the visible structures within the cortical units are asymmetrically placed, the patch of inverted kineties (*IK*) is bounded on its right side by an unusually wide space (*a*), and, on its left side, by an unusually narrow space (*b*) (see also Fig. 1). The tripartite, triangular granules (two-kinetosome units) point to the right in normally oriented kineties (*NK*), but to the left in the IK patch, showing the opposite polarity of cortical units in the two regions. The IK patch of 3B1 cells consists of 15–20 adjacent inverted rows, extending from about the 15th kinety to the left of the vestibule (*v*) around to the middorsal surface. *cvp*, contractile vacuole pore; *A*, anterior; *P*, posterior; *R*, right; *L*, left. × 1,250. *Inset* in 3 *a*: outlined area of the wide strip between NK and IK regions (see also Fig. 1, rows 1 and 2). This area was chosen for its high frequency of two-kinetosome units, although the wide stripe tends to be obliterated by the contour of the body in this region. Triangular granules representing two-kinetosome (*k*) units point away from each other on either side of the NK-IK boundary (dotted line), and are resolvable as tripartite with the point of the triangles representing the position of the parasomal sac (*s*). × 3,750. *Inset* in 3 *b*: outlined area of the narrow stripe between NK and IK regions (see also Fig. 1, rows 4 and 5). Tripartite triangular granules (two-kinetosome units) point towards each other on either side of the NK-IK boundary (dotted line). × 3,750.

inverted rows was the basis for abnormal swimming patterns, there was no direct evidence to indicate the reason for this behavior. The most obvious explanation was that the direction of ciliary beat was reversed on the inverted cortex. Nevertheless, alternative explanations, such as fewer cilia in the inverted patch, weaker beating of cilia in this region, or the differences in kinety spacing at the two sides of the inverted patch, could not be completely ruled out. Nor was it known whether the cilia in the IK region were capable of making directional responses to depolarizing stimuli. If they could not, then the 3B1 stock would be of only limited use for studying the role of cortical orientation in the directional responses of cilia.

We therefore used cinemicrography and scanning electron microscopy to determine whether cilia on the IK patch of forward *and* backward swimming 3B1 cells always beat in a direction opposite to that of normally oriented cilia.

Cinemicrography

The direction of ciliary beat in different regions of living cells was determined from the direction of flow of carmine particles near the cells in cine films.

Wild type controls swimming forward in a suspension of carmine particles in culture fluid created water currents directed uniformly backwards around the entire cell. This current pattern was seen most clearly in cells almost at rest against the substrate, where the force exerted by the beating cilia was used almost entirely to move fluid past the cell rather than to move the cell through the medium (Figs. 5, 8 *a*).

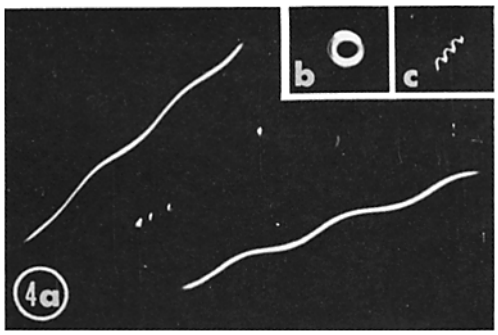


FIGURE 4 Swimming paths of paramecia in depression slides (darkfield, 1-s exposure). (*a*) Wild type cells swimming forward (Dryl's solution); (*b*) 3B1 cell swimming forward in circles (Dryl's solution); (*c*) 3B1 cell swimming backwards in a straight direction (K^+ solution). × 16.

In contrast, 3B1 cells swimming in a suspension of carmine in Ba^{++} solution showed a characteristic countercurrent pattern of particle movements during forward circling (Figs. 6, 8 *b*). On the NK side of the cell, carmine particles were swept posteriorly as in controls. However, on the left dorsal side of the cell where the IK patch is located, forward water currents were produced, indicating a reversal in direction of beat in this region.

Films of 3B1 cells swimming backwards in circles in the same Ba^{++}-carmine suspension showed that the countercurrent pattern of forward circling was reversed on both sides of the cell, i.e. the direction of water currents on the NK surface changed from backwards to forwards, while that on the IK side changed from forwards to backwards (Figs. 7, 8 *c*).

These current patterns were typical of 3B1 cells swimming forwards and backwards in culture medium (containing dense, bacterial suspension or carmine) as well as in the Ba^{++}-carmine suspension used for cine records. The results thus indicate that the cilia of the inverted rows always beat in a direction opposite to that of normally oriented cilia, during both forward *and* backward circling (Fig. 8).

Scanning Electron Microscopy

Scanning electron microscopy of cells fixed instantaneously while swimming revealed aspects of ciliary activity not shown by other methods. First, the ciliature of the IK patch is apparently normal with respect to the number and length of its cilia (Figs. 10–14). This finding virtually eliminated possible defects in ciliary density or length as explanations for the abnormal swimming behavior of 3B1 cells.

The form of ciliary beat and the pattern of metachronal coordination in wild type *Paramecium* have been described recently in instantaneously fixed cells by scanning electron microscopy (Tamm, 1972), and in living cells by flash photography (Machemer, 1972 *a–c*). Both methods of observation give similar results, showing that the scanning micrographs of ciliary activity faithfully reflect the living state.

In forward swimming wild type paramecia, the effective stroke is directed obliquely backwards, in a plane which is almost parallel to the metachronal

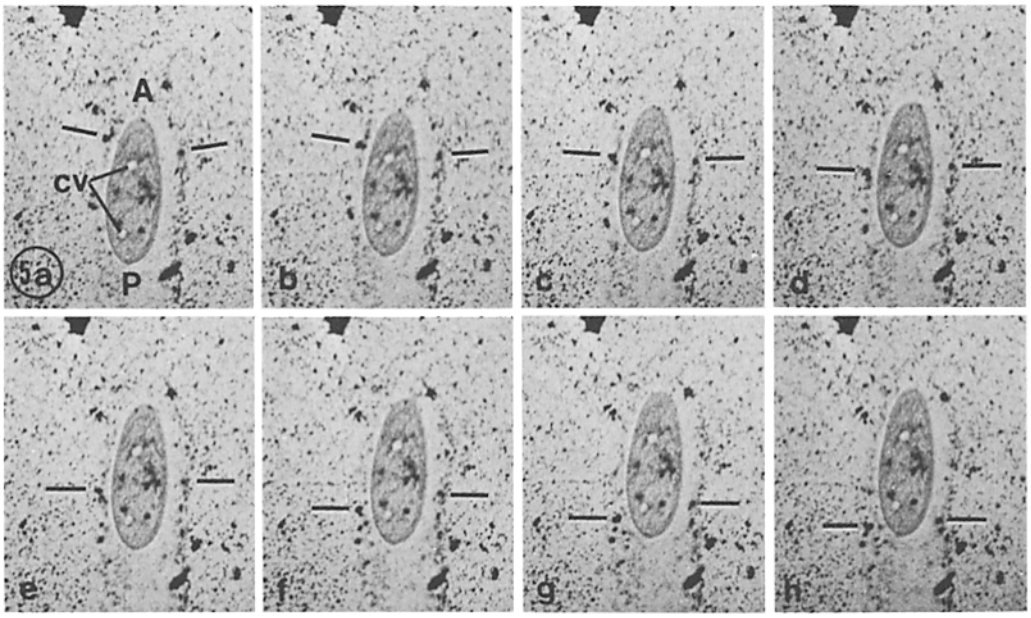


FIGURE 5 Cine prints of a wild type *Paramecium* swimming slowly forward (culture medium with carmine particles). The cell is slightly flattened by the coverslip. The dorsal surface of the cell faces the reader, so the side to the reader's left corresponds to the cell's left, and the edge to the reader's right is the cell's right side. Note that particles (marked by lines) are swept backwards on both sides of the cell, indicating a posteriorly directed beat around the entire cell. Time interval between successive frames (prints *a–h*) is 0.1 s. *A*, anterior; *P*, posterior; *cv*, contractive vacuole. × 150.

wave fronts (Fig. 9). The waves travel anteriorly at right angles to the direction of the effective stroke, resulting in a dexioplectic pattern of coordination (Knight-Jones, 1954; Machemer, 1972 *a–c*; Tamm, 1972). The recovery stroke of the beat cycle is a counterclockwise rotation (as viewed from outside the cell) close to the cell surface and out of the plane of the effective stroke (Fig. 9). Because changes in the direction of the effective stroke are accompanied by corresponding changes in the pattern of coordination (see Machemer, 1972 *c*), the orientation of the metachronal waves can be used as an indicator of beat direction. In any wave pattern, an arrow drawn parallel to the wavefronts and pointing towards the concave side of the curving recovery stroke cilia approximates the direction of beat (Fig. 9).

3B1 cells were fixed instantaneously during forward circling in culture fluid and during backward straight swimming in K^+ solution. In both types of locomotion a region on the left dorsal side of the cell was characterized by a normal form of ciliary beat and a normal pattern of ciliary coordination, but the *directions* of these parameters were reversed compared to the ciliary pattern on the rest of the cell (Figs. 10–14). This area of reversed polarity in ciliary activity corresponds to the location of the IK region as determined by silver-stained specimens prepared concurrently (Fig. 3).

For example, in forward circling 3B1 cells the effective stroke is directed obliquely backwards in the NK region (similar to its direction in forward swimming wild type cells) and forwards in the IK patch (Figs. 10–13). Conversely, 3B1 cells fixed during K^+-stimulated backward, straight locomotion show an anteriorly directed beat in NK areas but a posteriorly directed beat in the IK region (Fig. 14).

Scanning electron microscopy thus shows that the change from forward circling to backward, straight swimming is accompanied by ciliary reversal on IK as well as NK regions. This confirms the cine records of simultaneous reversals on both IK and NK sides during avoiding reactions of living 3B1 cells.

The angular difference in the direction of the effective stroke between the NK and IK regions can be readily estimated by comparing the orientation of the wavefronts in the two areas. The expected 180° difference in beat direction between

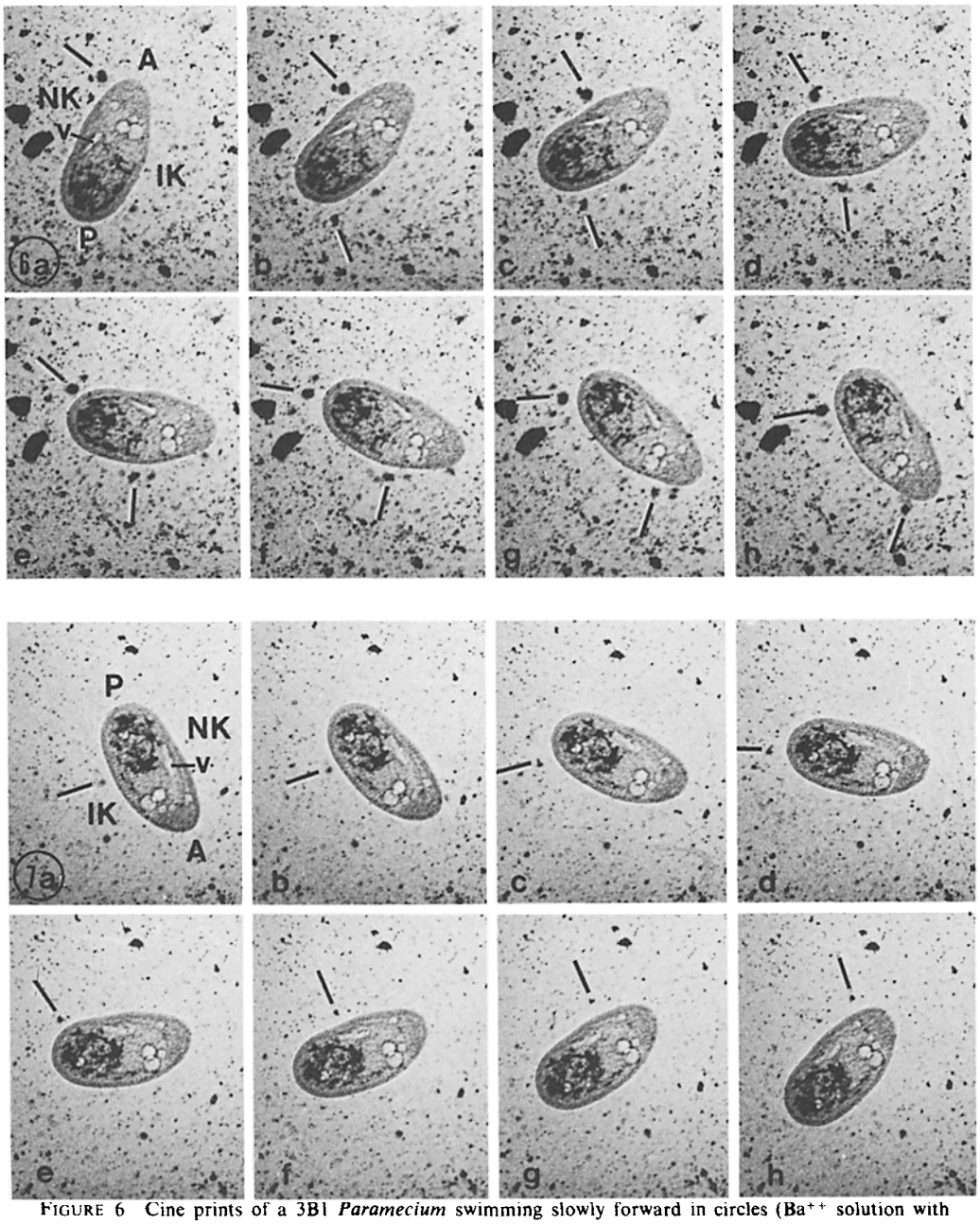


FIGURE 6 Cine prints of a 3B1 *Paramecium* swimming slowly forward in circles (Ba^{++} solution with carmine particles). The cell is slightly compressed, with its right ventral surface, consisting of normally oriented cortex (*NK*), facing the outside of the circular path. On this side, particles are swept from anterior to posterior (lines). On the other side, corresponding to the left dorsal surface where inverted kineties (*IK*) are present, particles are carried in the opposite direction, i.e., from posterior to anterior (lines). Time interval between successive frames (prints *a–h*) is 0.1 s. *A*, anterior; *P*, posterior; *v*, vestibule. × 150.

FIGURE 7 Cine prints of the same 3B1 cell shown in Fig. 6, but swimming backwards in circles in this sequence. Particle movements are exactly reversed on both sides of the cell compared to the pattern observed during forward circling (cf. Fig. 6), i.e., particles are carried from posterior to anterior on the NK side, and from anterior to posterior on the IK side (lines). Time interval between successive frames (prints *a–h*) is 0.1 s. *A*, anterior; *P*, posterior; *v*, vestibule. × 150.

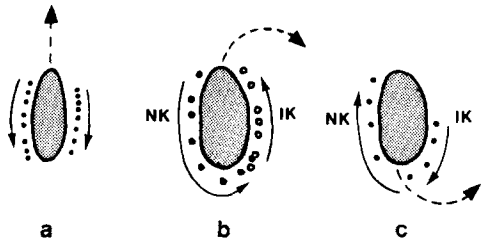


FIGURE 8 Directions of carmine particle movements near (*a*) forward swimming wild type *Paramecium*, (*b*) forward circling 3B1 cell, and (*c*) backward circling 3B1 cell. The cells are those shown in Figs. 5–7, respectively, with the positions of the marked particles in successive frames (*a–h*) traced from the cine prints. Solid arrows show direction of particle movements relative to normally oriented (*NK*) and inverted (*IK*) cortical regions. Dashed arrows indicate direction of cell movement. The cells are oriented with their anterior ends at the top of the figure.

the NK and IK regions is shown for forward swimming (Fig. 10) and backward swimming 3B1 cells (Fig. 14). In some forward circling cells (Figs. 11, 12), however, the directions of beat in the two cortical regions are not exactly 180° out of phase. This is because the effective stroke in the IK region of these cells is directed almost straight forward, instead of obliquely forward. As a result, the wavefronts in the IK patch are oriented approximately parallel to the cell's antero-posterior axis. The direction of beat in the NK region is similar to that in wild type controls—i.e., obliquely posterior. Nevertheless, the kineties of the IK and NK regions run parallel to one another, and thus are reversed exactly 180°, in all 3B1 cells. The reason for this small discrepancy between kinety orientation and beat direction in the IK patch of some cells is not yet understood.

The wave pattern on a typical backward straight swimming 3B1 cell shows that the opposing directions of beat in both NK and IK regions are almost parallel to the cell's antero-posterior axis (Fig. 14). Such a large shift in beat direction is characteristic of a strong avoiding reaction.

Scanning electron micrographs also reveal that well-defined (but oppositely oriented) waves of ciliary coordination are present immediately adjacent to the borders of the NK and IK regions. Figs. 10 14 show the sharp demarcation in wave pattern that characterizes the junction between oppositely oriented cortical regions.

In contrast to the area of reversed beating found on the left side of 3B1 cells, wild type controls show no such reversal in ciliary activity at corresponding sites on the cortex (Fig. 9).

Occasionally, 3B1 cells were observed in which ciliary coordination was poor or apparently lacking in the IK region, but normal on the rest of the cell. If these cases represent real differences in ciliary activity, it is possible that opposing hydrodynamic forces disrupted the pattern of coordination on the IK region. Recent work indicates that the cilia of *Paramecium* are coordinated by mechanical interaction (Machemer, 1972 *a–c*; Naitoh and Kaneko, 1972, 1973). Since the cilia of the inverted patch are being carried in the direction opposite to that in which they would tend to move the cell, they may experience mechanical forces that interfere with their coordination.

Behavior of 3B1 Stock Wild Type for Paranoiac

The 3B1 cells used for the majority of this work were homozygous for paranoiac (Pa), a behavioral mutation which causes overreaction to Na^+-rich solutions (Kung, 1971 *a*). Theoretically, the presence of Pa in the 3B1 stock should not have affected the relation between the directions of ciliary beat in NK *vs.* IK regions. Nevertheless, we used a 3B1 stock wild type for Pa (3B1 Pa^+) to repeat certain behavioral observations described above in order to rule out this possibility.

In culture medium the 3B1 Pa^+ cells swam forward in circular paths as did our original 3B1 stock. In carmine suspension, 3B1 Pa^+ cells showed the same countercurrent pattern of particle movements during forward circling as illustrated for 3B1 with Pa in Fig. 6, and the same reversal of this pattern on both sides during backward circling (cf. Fig. 7). K^+ solutions stimulated 3B1 Pa^+ cells to swim backwards in straight paths, as did the original 3B1 stock.

These behavioral observations indicate that, as expected, the paranoiac mutation has no influence on the relationship between the directions of beat in NK *vs.* IK cortex and thus can be ignored in the interpretation of our results.

DISCUSSION

This investigation set out to answer a basic question about the directional control mechanism in cilia with a variable plane of beat: is the reference for the directional response of the cilia to stimuli located within the cortical structures themselves, or is it a property of the organism as a whole?

To answer this question, we determined the relation between the direction of beat and the orientation of the ciliary rows in mosaic 3B1 paramecia containing both normally oriented (NK) and inverted (IK) cortical regions. Cine films of water currents produced by living 3B1 paramecia, as well as scanning electron microscopy of instantaneously fixed cells, showed that the cilia of the inverted cortex always beat in the direction opposite to that of the cilia on the rest of the cell. This finding explains the abnormal swimming patterns of paramecia with inverted kineties.

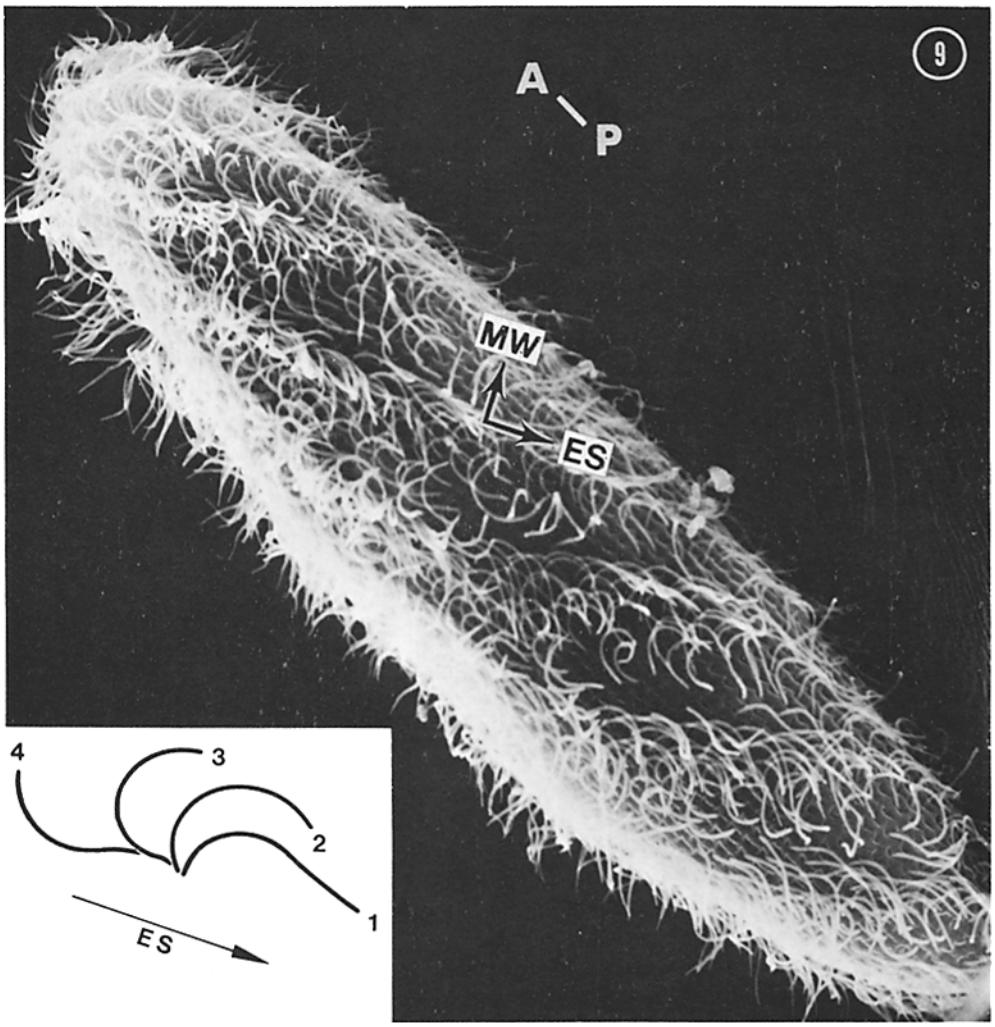
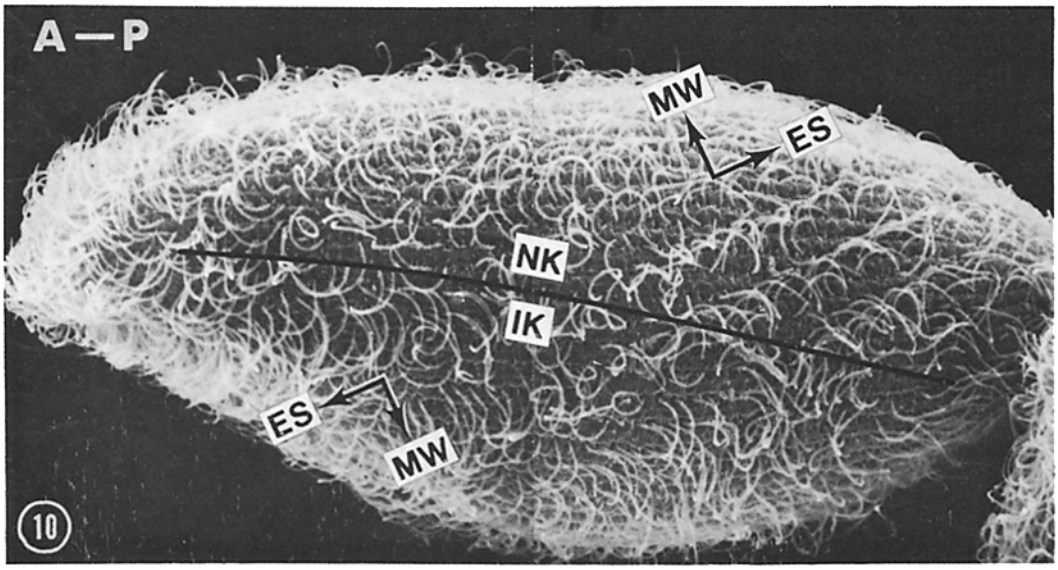
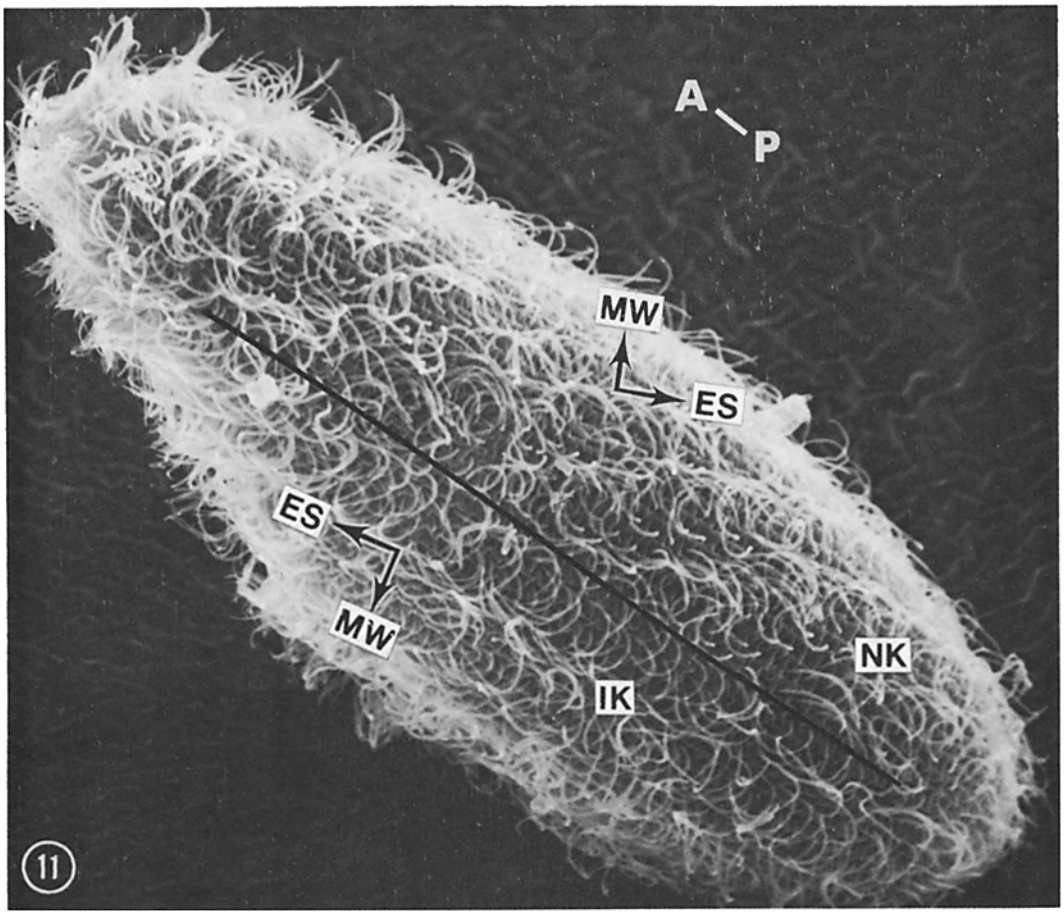


FIGURE 9 Scanning electron micrograph (SEM) of the left dorsal surface of a wild type *Paramecium* fixed during forward swimming in culture medium. The effective stroke (*ES* arrow) of all cilia is in an obliquely posterior direction, approximately parallel to the metachronal wave fronts which travel obliquely anteriorly (*MW* arrow), at right angles to the direction of beat. Successive stages in a complete beat cycle of a cilium are encountered as one goes from anterior to posterior through one metachronal wave. *Inset* shows the form of beat of a single cilium. Numbers refer to consecutive stages in the beat cycle: (1) at end of effective stroke; (2–3) counterclockwise rotation during the recovery stroke; (4) at end of the recovery stroke and preparatory to the effective stroke. The effective stroke (*ES* arrow) takes place from position 4 to position 1, out of the plane of the paper. Note that in this micrograph and the following SEM figures, an arrow drawn parallel to the wavefronts and pointing towards the concave sides of the curving recovery stroke cilia can be used as an indicator of the direction of beat. *A-P*, antero-posterior axis. × 1,530.


A—P
MW
ES
NK
IK
ES
MW
10


A
P
MW
ES
ES
MW
NK
IK
11

More importantly, we found that depolarizing stimuli which caused changes in beat direction of NK cilia induced a corresponding but oppositely directed change in beat direction on the inverted cortex. The functional orientation of the cilia is therefore rotated along with the morphological orientation of the cortex.

These results demonstrate that the reference point for the directional response of protozoan cilia with a variable plane of beat resides within the cilia or their immediate cortical surroundings. Since other cortical structures besides cilia and basal bodies are reversed in the IK region of 3B1 paramecia, a more precise localization of the reference point is not possible at this time.

A surprising behavioral finding was the straight path, instead of circular movement, taken by backward swimming 3B1 cells in response to K^+ and Na^+ solutions. Scanning electron microscopy showed that the straight, backward paths are not due to failure of the IK cilia to reverse beat direction (see Fig. 14). Instead, straight, backward swimming probably represents a stronger reversal response, in which the direction of effective stroke is shifted maximally, i.e., directly anterior in the NK region and directly posterior in the IK region (Fig. 14, and unpublished results). This would maximize the components of force exerted by NK and IK cilia along the antero-posterior axis, thus increasing the absolute difference between them and favoring the action of the larger NK region; this, in turn, should result in a more normal (i.e. straight) swimming path.

Developmentally, beat direction is determined differently in *Paramecium* cilia compared to metazoan ciliary systems. Reversed polarity grafts done on amphibian embryo epithelium *before* the outgrowth of cilia showed that the fixed plane of beat is determined by the polarity of the embryo as a whole (Twitty, 1928). Since the 3B1 paramecia used in our study were descended (through hundreds of cell fissions) from a cell possessing a cortical rearrangement, the functional polarity of newly formed cilia in *Paramecium*, like the spatial organization of new cortical structures (Sonneborn, 1963; Beisson and Sonneborn, 1965; Sonneborn, 1970 *a*; Dippell, 1968), is determined by the preexisting cortical pattern.

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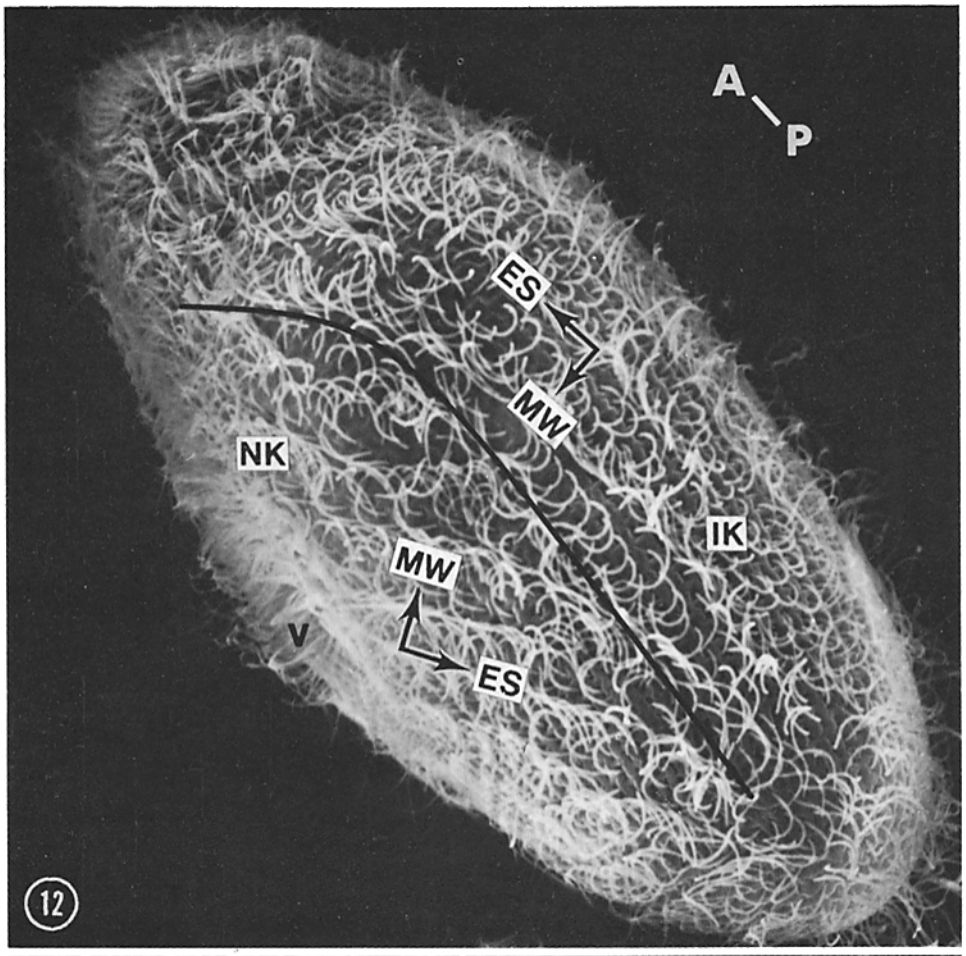
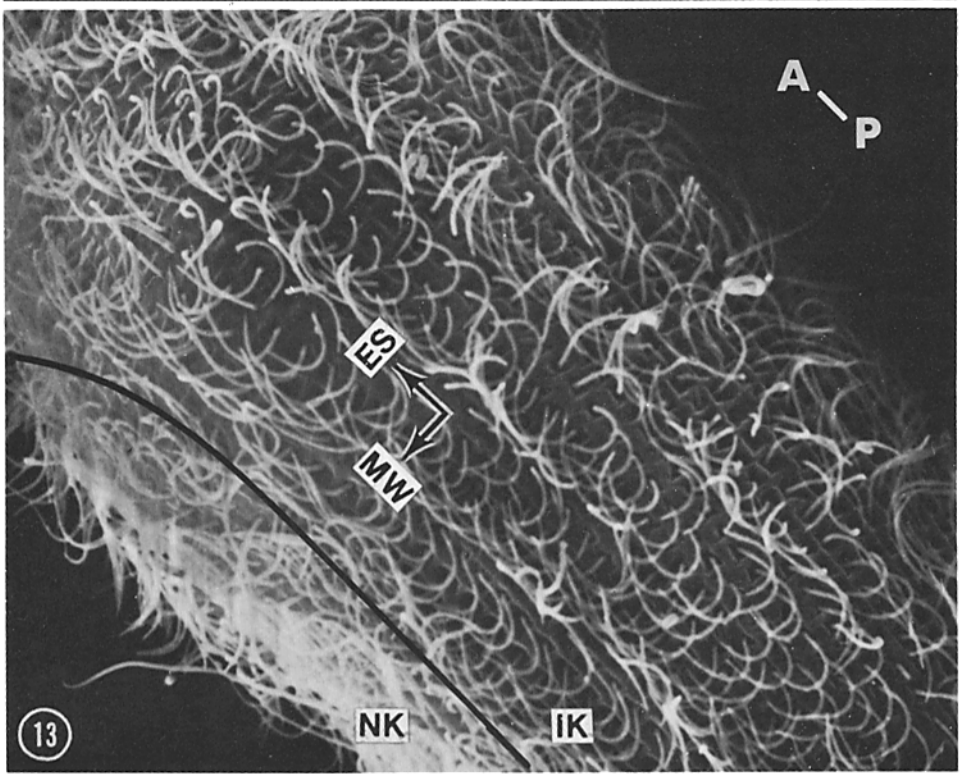
FIGURE 10 SEM of the left dorsal surface of a 3B1 *Paramecium* fixed during forward circling in culture medium. Line drawn on the cell indicates the border between regions with opposite patterns of ciliary activity. This line corresponds to the left boundary of the inverted kinety patch (*IK*), as determined by silver-stained preparations of similar material (Fig. 3 *b*). On normally oriented cortex the effective stroke is directed obliquely backwards (*ES* arrow, *NK* region), and metachronal waves travel obliquely forwards (*MW* arrow, *NK* region), as in forward swimming wild type cells (Fig. 9). On the IK patch these parameters are almost exactly reversed: the effective stroke is directed obliquely forwards (*ES* arrow, *IK* region) and metachronal waves travel obliquely backwards (*MW* arrow, *IK* region). The reversal in polarity of ciliary activity is readily apparent by comparing the directions of curvature of recovery stroke cilia in the NK vs. IK regions: the inner concave sides of these cilia point backwards in the NK cortex and forwards in the IK patch (see also Figs. 11–14). Note that there is no apparent difference in ciliary number or ciliary length between the NK and IK regions (see also Figs. 11–14). *A-P*, anterio-posterior axis. × 1,380.

FIGURE 11 SEM of the left dorsal surface of another 3B1 *Paramecium* fixed during forward circling in culture medium (cf. Fig. 10). As in Fig. 10, the line marks the left boundary between the inverted kinety patch (*IK*) and normally oriented cortex (*NK*). Well preserved waves of ciliary coordination are present on both NK and IK regions. The effective stroke is directed almost straight forward instead of obliquely forward on the IK patch, resulting in less than a complete 180° reversal in beat direction between the NK and IK regions (see Fig. 10 for explanation of symbols). × 1,640.


A
P
ES
MW
NK
IK
MW
V
ES
12


A
P
ES
MW
NK
IK
13

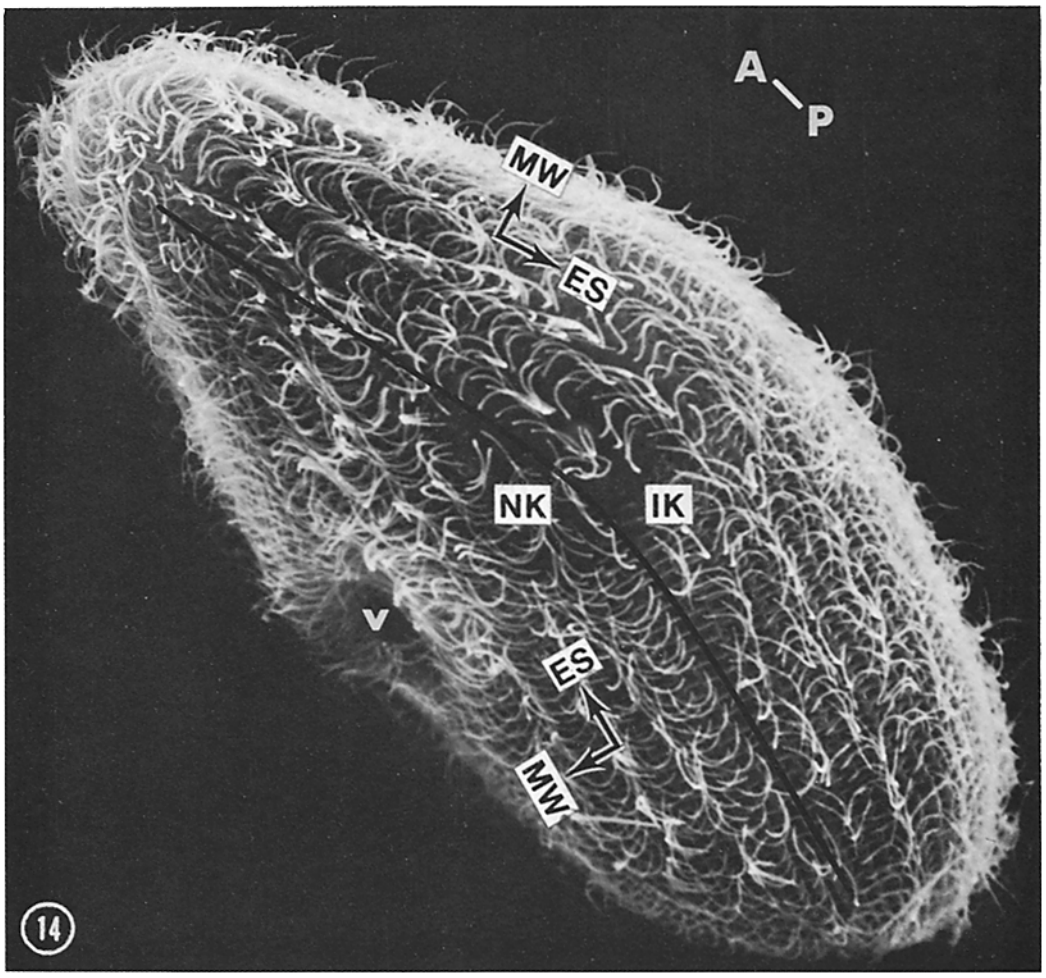


FIGURE 14 SEM of the left ventral surface of a 3B1 *Paramecium* fixed during backward straight locomotion in K^+ solution. The directions of effective stroke and metachronal wave transmission on both NK and IK regions are reversed compared to their pattern on forward circling 3B1 cells (see Fig. 12.). Note that the direction of beat on the NK region is now parallel, not oblique, to the cell's antero-posterior axis. *v*, vestibule (see Fig. 10 for explanation of symbols). × 1,330.

FIGURE 12 SEM of the left ventral surface of a 3B1 *Paramecium* fixed during forward circling in culture medium. Line drawn on the cell marks the border between regions with opposite polarity of ciliary activity, and corresponds to the right boundary of the inverted kinety patch (*IK*) as determined by silver-stained preparations of similar material (Fig. 3 *a*; note normal curvature of kineties on the anterior left ventral surface). The effective stroke is directed obliquely backwards on the NK region and almost straight forwards on most of the IK patch. *v*, vestibule (see Fig. 10 for explanation of symbols). × 1,370.

FIGURE 13 Same 3B1 cell as in Fig. 12, but tilted to the right and enlarged to show the ciliary pattern on the IK patch in greater detail. Note that the form of ciliary beat and the pattern of metachronal coordination on the IK patch are normal, but reversed in direction (see Fig. 10 for explanation of symbols). × 1,980.

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