zation would lend support to the masked mRNA hypothesis, whereas a contrary finding would reinforce the hypothesis that mRNA already in use in the egg becomes more fully saturated with ribosomes when fertilization occurs and is the factor primarily responsible for the increased rate of protein synthesis.

For analysis of the proteins made under various conditions, it seemed desirable to find a method for extracting as large as possible a fraction of the sea urchin egg and embryo protein, especially because extracts containing less than 5 percent of the proteins of the egg had been previously used (*9, 14*). We did not use buffers of high salt concentration to solubilize the protein, even though they might be expected to work well with cells of the sea urchin, which is a high salt organism (*15*), because use of disc electrophoresis constrained us to the use of gels of low salt concentration. Use of urea and nonionic detergents, however, proved an effective way of solubilizing the sea urchin proteins. Table 1 shows the efficiency of entry into the gel of radioactive material in the homogenate under a variety of extraction conditions, the optimum yielding an efficiency of about 50 percent.

The pattern of stained protein in the gels did not vary perceptibly from one group of eggs to another. They did vary depending upon the procedure used for extraction of proteins and the conditions of labeling (short or long exposure, or exposure to isotopic precursor followed by exposure to unlabeled precursor) so that legitimate comparisons can be made only among gels of the same group. Because of the high density of bands it was not practical or meaningful to assign specific peaks of radioactivity to specific stained bands. Instead, autoradiogram tracings were compared directly. In three groups of gels (Fig. 1) the spectrum for the unfertilized egg is similar to that for the newly fertilized egg or that of the unfertilized egg in which protein synthesis had been stimulated. The patterns are complex, but each peak in one spectrum has a corresponding peak or shoulder in every other.

Comparisons of proteins made in unfertilized and newly fertilized eggs are shown by actual tracings of autoradiographs of the gels (Fig. 2). Significant differences cannot be observed, nor are they apparent when comparing autoradiographs of gels containing proteins from eggs stimulated by CO_2 removal or by prior anaerobiosis. However, proteins from gastrula-stage embryos extracted in 8*M* urea (1 percent Brij) do differ substantially from proteins in the unfertilized egg or in the zygote (Fig. 1); this demonstrates that our extraction procedure does not extract only some fundamental class of proteins which are synthesized at all stages and which constitute a substantial fraction of the total protein synthesized.

The possibility that new proteins are in fact made immediately after fertilization in small quantity cannot be ruled out by the foregoing experiments, even if coincidence of peaks is taken to imply identity of proteins. The actual peaks represent no more than 10 percent of the total synthesis which occurs in these eggs, the remainder of the 50 percent of the radioactivity of the homogenate which entered the gel being distributed as radioactivity not resolvable as bands throughout the gel. The possibility that new proteins are represented cannot be excluded except by an exhaustive fractionation and high-resolution analysis of all the protein of the egg and embryo. That major changes in the pattern of protein synthesis have not occurred is consistent with the view that peptide chain initiation is responsible for most of the rate of change at fertilization. Monroy (*16*) finds no change in the average size of peptide chains made before and after fertilization in *Paracentrotus lividus* (comparison based on density-gradient centrifugation in sodium dodecyl sulfate sucrose) which is also consistent with this view, as are the findings of Whiteley, McCarthy, and Whiteley (*17*) who show by molecular hybridization that mRNA's from unfertilized eggs and blastulas are not distinguishable.

F. ROY MACKINTOSH
EUGENE BELL
Department of Biology, Massachusetts Institute of Technology, Cambridge

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Ionic Mechanisms Controlling Behavioral Responses of Paramecium to Mechanical Stimulation

Abstract. *A mechanical stimulus applied to the anterior part of* Paramecium *causes a transient increase in membrane permeability to calcium. This permits a calcium current to flow into the cell, causing the membrane potential to approach the equilibrium level for calcium. The transient depolarization which results elicits a reversal in the direction of ciliary beat. When the organisms are free-swimming this is seen as the reversed locomotion of Jennings' "avoiding reaction." In contrast, a mechanical stimulus applied to the posterior part results in increased permeability to potassium ions, and hence an outward potassium current. The hyperpolarization which results causes an increase in the frequency of ciliary beat in the normal direction. In free-swimming specimens this is seen as an increase in the velocity of forward locomotion.*

The varieties of locomotor behavior exhibited by *Paramecium* depend on relative changes over the cell surface in the frequency, strength, and orientation of ciliary motion (*1–3*). When the paramecium encounters a noxious chemical or a mechanical stimulus with its anterior end, it transiently reorients (reverses) its cilia, which causes it to swim backward for a short distance.

It then pivots aborally about its posterior end, due to the anatomical sequence with which forward-swimming ciliary orientation is restored, and resumes forward locomotion in the new direction (*4*, *5*). A full avoiding reaction requires a concerted reversal of cilia over the entire surface of the cell (*2*).

What is the mechanism by which a localized stimulus signals the ciliary apparatus of the entire cell to undergo a reversal in the direction of beat? Previous studies indicate that ciliary reversal is calcium and adenosine triphosphate (ATP) dependent (*6*), and is initiated by a reduction of the membrane potential (*7*, *8*). We postulated that a stimulus applied to one part of the organism causes a depolarization which spreads as a result of the electrical properties of the cell. This hypothesis was tested as follows.

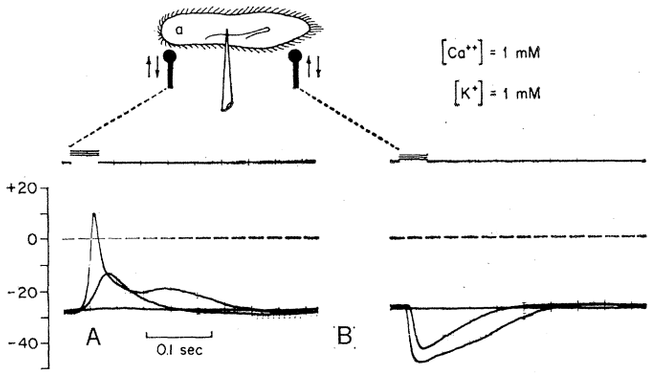
Single specimens of *Paramecium caudatum* were mechanically immobilized (*9*), in solutions containing $CaCl_2$ and KCl in 1 m*M* tris(hydroxymethyl) aminomethane–HCl buffer (*p*H 7.2 to 7.4). Intracellular recordings were made with conventional 3-M KCl capillary electrodes of about 20-megohm tip resistances. The recording electrode was inserted in the midsection of the cell; however, the location of the electrode did not have any effect on the recorded signal because the cytoplasmic compartment of *Paramecium* is essentially isopotential (*10*). Mechanical stimuli were applied to selected portions of specimens by means of a crystal-driven glass stylus with a tip diameter of 25 μ. The crystal was activated by 50-msec voltage pulses from a pulse generator synchronized with the oscilloscope sweep.

Responses to mechanical stimulation were transient potential changes (receptor potentials). The polarity of the receptor potential depended on the anatomical region of stimulus application. Stimulation of the anterior part elicits a depolarization, whereas stimulation of the posterior part elicits a hyperpolarization (Fig. 1). Both types of responses are continuously graded according to stimulus intensity (stylus displacement) to maximum levels which in both cases are functions of the ionic environment, as described below. The anterior and posterior receptor potentials differ both in their time course and their sensitivity. The posterior receptor potential shows the greater sensitivity for low-level stimuli. Its maximum response occurs at lower intensities of stimulation, and within that range of intensities shows more pronounced deviations from the resting potential than exhibited by the anterior receptor potential. Stimuli applied to the midsection of the cell do not elicit electrical responses. Stylus excursions of 3 to 4 μ applied to the membrane of the anterior elicit depolarizing responses of about one half the maximum amplitude. Posterior receptor potentials can be detected in response to hydraulic pressure waves set up by excursions of the stylus with the stylus positioned up to 50 μ from the posterior surface of the specimen.

The consistency of the electrical responses, the correlation of both wave shape and polarity with locus of stimulation, and the failure of electrical responses in deteriorated specimens dispenses with any likelihood that these potentials are physical artifacts of movement.

By what means do mechanical stimuli applied to different regions of a continuous cell membrane result in changes in membrane potential of opposite polarity? The ionic hypothesis of membrane potentials (*11*) suggests that both the anterior and the posterior potentials are generated by (i) selective permeability changes of the membrane in response to stimulation, (ii) flow of permeant ions across the membrane down their electrochemical gradients, and (iii) the resulting current causing the potential across the membrane capacitance to approach the equilibrium potentials for the permeant ions. Changes in the concentration gradients of the permeant ions across the cell membrane alters the potential levels at which they are in electrochemical equilibrium across the membrane, and hence should alter the potential levels approached by the receptor potentials.

To test this hypothesis receptor potentials were monitored in specimens bathed in solutions of various concentrations of calcium and constant concentration of potassium ([K^+]) (Fig. 2A). Peak potential values in response



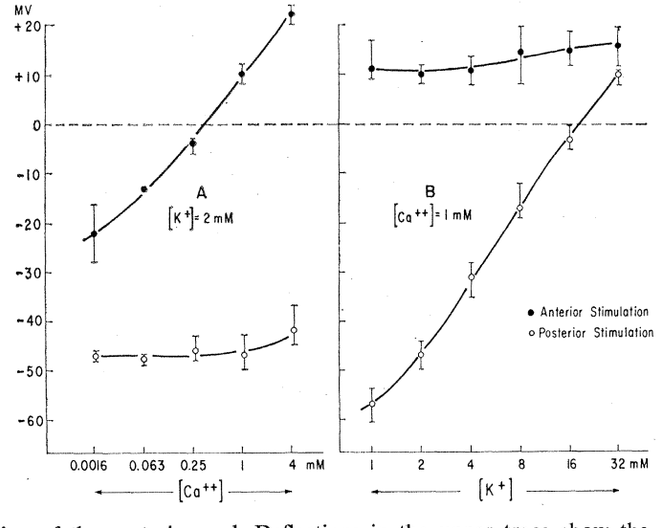


Fig. 1 (left). Mechanoreceptor potentials in *Paramecium*. Diagram shows an immobilized specimen impaled by an intracellular recording electrode. Mechanical stimuli of varying displacements were applied to either the anterior (*a*) or the posterior regions of the cell. (A) Anterior receptor potentials elicited by three intensities of mechanical stimulation of the anterior end. (B) Posterior receptor potentials elicited by mechanical stimulation of the posterior end. Deflections in the upper trace show the duration and relative intensity of pulses activating the piezoelectric crystal. Fig. 2 (right). Peak values of receptor potentials plotted against Ca^{++} and K^+ concentrations. (Ordinate) Intracellular potential recorded at the peak of the anterior receptor potential (●) and the posterior receptor potential (○). In graph A [K^+] was held at 2 mmole/liter, and in graph B [Ca^{++}] was held at 1 mmole/liter. Vertical bars show range of data from 5 to 12 measurements on as many different specimens. Only maximum responses were used. A maximum response is defined as one which shows no increase with further increase in stimulus intensity.

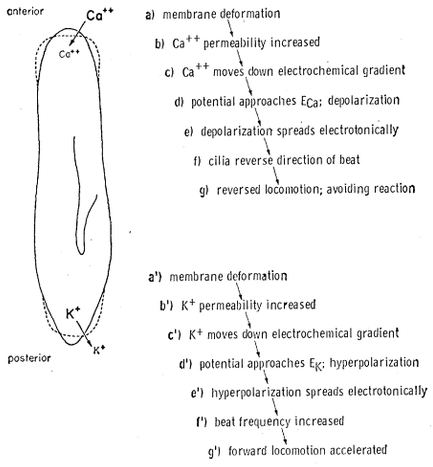


Fig. 3. Summary of steps linking mechanical stimuli with motor reactions of *Paramecium*. The anterior and posterior ends of the organism are shown at the top and bottom, respectively. Major steps leading to either the avoiding reaction or accelerated forward locomotion are outlined near the end of the cell which when stimulated initiates the sequence. The molecular mechanisms involved in steps a, a′, f, and f′ are not known.

to both anterior and posterior stimulation were plotted as functions of the calcium concentration ($[Ca^{++}]$). This was repeated at constant $[Ca^{++}]$ in a series of potassium concentrations (Fig. 2B).

The peak of the anterior receptor potential (closed circles) showed a slope of 22 mv for a tenfold change in $[Ca^{++}]$; the peak of the posterior receptor potential (open circles) showed a slope of 50 mv for a tenfold change in $[K^+]$. These values approach the theoretical slopes of 29 and 58 mv respectively for Ca^{++} and K^+ diffusion potentials at room temperature. Finally, the peak value of the posterior receptor potential (closed circles) is independent of $[Ca^{++}]$, whereas the peak of the anterior receptor potential (open circles) is independent of $[K^+]$ (Fig. 2).

These results lead to the conclusion that a mechanical stimulus to the anterior end induces a transiently increased membrane conductance to Ca^{++}, whereas a similar stimulus to the membrane of the posterior end causes a transiently increased membrane conductance to K^+. As a result, stimulation of the anterior end causes a transient approach of the membrane potential toward the equilibrium level for Ca^{++}, and stimulation of the posterior end a transient potential shift toward the equilibrium potential for potassium.

The external concentrations of calcium and potassium determine their transmembrane electrochemical gradients and hence influence the magnitude and direction of the receptor potentials (Fig. 2) as well as the value of the resting potential (*9*). We chose the concentrations in the standard mediums (Fig. 1) arbitrarily, but they are in the range typical of natural habitats of *Paramecium*.

Parallels between electrical behavior and locomotor behavior are consistent and complete (Fig. 3). For example, the avoiding reaction is increased as the intensity of the stimulus is increased (*1*). This parallels both the increased intensity of ciliary reversal with increased depolarization (*8, 12*) and the increased depolarization as submaximal mechanical stimulation is increased (Fig. 1). Jennings (*5*) also noted that the avoiding reaction occurs only to mechanical stimulation of the anterior end. Hyperpolarization in both *Paramecium* and *Opalina* is accompanied by an increased rate of ciliary beat in the forward-swimming direction (*7, 8, 12*). This parallels the transient increase in swimming rate which occurs in response to a general mechanical stimulus such as a sharp tap applied to the culture vessel (*3*). The dominance of the posterior receptor potential in the case of a generally applied stimulus may result from the greater mechanical sensitivity of the posterior end noted in our experiments.

These findings appear to be unique in demonstrating a direct relation between the coordinated locomotor behavior of an organism and ionic mechanisms of membrane excitability.

YUTAKA NAITOH*
ROGER ECKERT

Department of Zoology,
University of California, Los Angeles

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Microvolt Electric Signals from Fishes and the Environment

Abstract. *Pulses in the 0.01 to 40 microvolt range, probably generated by white fiber muscle action potentials, were remotely received through dipole antennae from five fishes and one amphibian in aquarium tests. In natural environments, however, no biologically generated signals have been detected. Received instead were a multitude of similar signals originating from unknown sources. The dominant types of these "atmospheric" signals and their reception rates change diurnally and can easily be confused with the fish-generated signals.*

Intriguing stories about strange signals produced by fishes, based on the work of W. L. Minto, Jr., and his associates, have recently appeared in popular magazines and trade journals (*1*); and Minto and Hudson (*2*) list 130 fishes that emit species-specific signals receivable with dipole antennae in aquarium tanks and, in some cases, the fishes' natural environment. Because the signals are said to be propagated through several hundred meters of water, these workers state that the signals are a novel energy form which they call "hydronic radiations."

Others (*3*) have studied the physics of the question. Here we confirm that by the use of equipment similar to Minto's, signals are receivable from fishes in laboratory tanks. These, however, are extremely weak electric pulses (10^{-14} watt into 500 ohms) most likely resulting from action potentials of white muscle fibers. Further, our field observations indicate that while such fish-generated signals may be receivable at close ranges in their natural environments, the majority of similar signals picked up in large bodies of water are electrically coupled components of ground currents and atmospheric noise originating from physical causes and are not generated by fishes.

Using the equipment shown in Fig. 1, we monitored and recorded numerous signals in freshwater tanks con-