ably distended. This enlargement of body cells is identical to that reported by Josephson and Macklin (*6*) when the internal gut pressure was increased to 4 to 6 mm of water.

Blanquet and Lenhoff (*5*) observed that tyrosine, when placed in the gut of a hydra, caused a neck to form if glutathione was present in the external environment. Since glutathione specifically elicits the feeding response in hydra and tyrosine is present in the gut because of the breakdown of digested prey, this neck-forming response was suggested to be an adaptive mechanism on the part of the hydra to prevent it from losing its gut contents when ingesting a second prey. We can now suggest why this mechanism is necessary. When a hydra is digesting its prey, its gut becomes turgid because of filling with fluid, and tyrosine in the gut prevents the mouth from opening by increasing the gut pressure required to trigger enteron-emptying contractions. This permits the animal to maintain a higher than normal gut pressure during digestion. After the prey is digested, whatever gut pressure is an adequate stimulus to cause a hydra to contract and expel the gut contents now becomes effective. Thus, tyrosine in the gut is probably not only a stimulus for neck formation but may also modulate the spontaneous contractile pulses (CP's) which could expel the gut contents. The behavior is identical in both cases and, therefore, it might be reasoned that neck formation is related to the mechanism which triggers enteron-expelling contractions.

The turgid condition of the hydra gut has been noted by other workers. Blanquet and Lenhoff (*5*) reported that the animals became turgid just after feeding. Marshall (*4*) reported that tying off an animal just below the tentacles results in the gut cavity becoming turgid. And it is a common observation of workers studying regeneration in hydra that regenerates frequently are noted to be turgid prior to the formation of a new mouth. This is particularly noticeable in regenerates that fail to regenerate a new mouth.

It is now clear how hydra eliminate excess gut fluid. The normal intact animal excretes excess fluid to the environment by opening its mouth and contracting. The presence of tyrosine in the gut is presumably inhibitory to mouth opening, and the stimulus for excreting the fluid is probably enteron pressure. For regenerates that do not have a mouth the response is identical. As the gut becomes distended the internal gut pressure increases and the animal contracts to expel the excess fluid. Since there is no mouth or other natural opening, the contractions may be sufficiently strong to burst the body wall.

MARTIN MACKLIN
THOMAS ROMA, KEVIN DRAKE
Department of Biomedical Engineering, Case Western Reserve University, Cleveland, Ohio 44106

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Calcium-Induced Ciliary Reversal in the Extracted Models of "Pawn," a Behavioral Mutant of Paramecium

Abstract. *"Pawn," a genic mutant of* Paramecium aurelia, *cannot swim backward as the wild type can upon proper stimulation. In contrast, after membrane disruption by Triton X-100, the adenosine triphosphate–magnesium reactivated models of Pawns swim backward in the presence of calcium as wild-type models do. Thus, the mutant phenotype is due to an impairment in the membrane and not in the calcium-sensitive motile system.*

The locomotor behavior of ciliate protozoans is largely dependent on a reversal of the direction of the effective power stroke of the cilia (ciliary reversal), which occurs in response to stimuli and causes the cells to swim backward (*1*). Recent evidence (*2*) indicates that ciliary reversal occurs in close association with a membrane depolarization which is mediated by an influx of Ca^{2+} in response to a transient increase in the calcium conductance of the membrane. An increase in cytoplasmic calcium concentration caused by the calcium influx activates a certain calcium-sensitive motile system which governs the orientation of ciliary beating (*3*, *4*).

Mutants of *Paramecium aurelia*, called "Pawn," however, fail to swim backward even in the face of stimuli such as collision with an obstacle and high potassium concentration, to which wild types respond by swimming backward (*5*). This behavioral deficiency in the Pawn can be explained by (i) a defect in the mechanism of calcium conductance increase in the membrane, or (ii) a defect in the calcium-sensitive reversal mechanism in the ciliary motile system, or by both. Recent electrophysiological studies of the Pawn membrane demonstrated a loss of excitability (that is, failure to exhibit an increase in calcium conductance in response to depolarization) (*6*). However, it is still desirable to determine whether any pleiotropic effect in the ciliary mechanism is involved in these mutants. We therefore examined the effect of calcium on the ciliary orientation of adenosine triphosphate (ATP)–Mg^{2+} reactivated,

Table 1. Ciliary reactivation of triton-extracted models of *Paramecium aurelia*. No significant differences in the reactivations were observed between "wild type" (strain 51s) and the mutant "Pawn" (strain d4-95).

Test substances*	Ciliary beat	Ciliary orientation†	Locomotion
EDTA	No	Posterior	No
Mg + EGTA	No	Posterior	No
Ca	No	Posterior	No
ATP + EDTA	No	Posterior	No
ATP + Mg + EGTA	Yes	Posterior	Forward swimming
ATP + Ca	No	Anterior	No
ATP + Mg + Ca	Yes	Anterior	Backward swimming

* Test substances were added into a basic 50 m*M* KCl solution buffered by 10 m*M* tris-maleate (*p*H 7.2 by NaOH). Concentration of each test substance was as follows: ethylenediaminetetraacetic acid (EDTA), 2 m*M*; Mg, 4 m*M*; ethylenebis(oxyethylenenitrilo)tetraacetic acid (EGTA), 3 m*M*; Ca, 10^{-6} *M*; ATP, 4 m*M*. † Ciliary orientation refers to the direction in which nonbeating cilia point or the direction of effective power stroke in beating cilia.

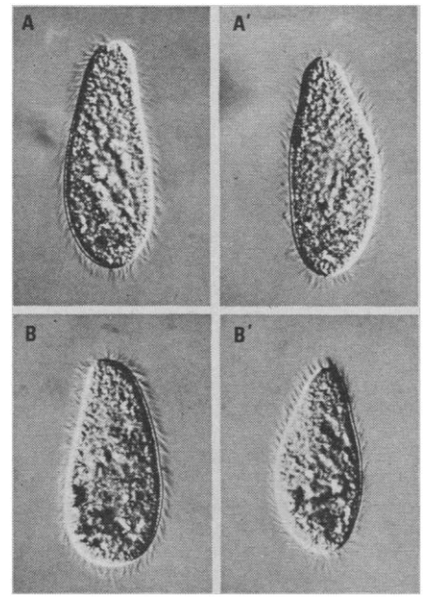

Fig. 1. Triton-extracted models of *Paramecium aurelia*. (A, A') Wild type (strain 51s). (B, B') A behavioral mutant "Pawn" (strain d4-95). (A, B) Models in 50 m*M* KCl solution with 2 m*M* ethylenediaminetetraacetic acid. Pointing direction of cilia is toward the rear (lower end of each photograph). Addition of $MgCl_2$ (4 m*M*) and ATP (4 m*M*) causes ciliary beat, hence the models swim forward. (A', B') Models in a KCl solution with 4 m*M* ATP and 10^{-6}*M* $CaCl_2$. Ciliary shafts are reversed to point anteriorly. Upon an addition of $MgCl_2$, models swim backward owing to reactivated ciliary beat in the reversed direction. Photographed under Zeiss-Nomarski interference-contrast illumination (× 220).

detergent-extracted models of the mutant Pawn, in which the cell membrane was functionally disrupted (*4*). In such models externally applied calcium has direct access to the cell interior, and can therefore influence the function of the ciliary apparatus without restriction by the surface membrane.

The models of Pawn swam backward when the calcium concentration in the ATP-Mg reactivation medium was raised above 10^{-6}*M*. This result shows that the reversal mechanism present in cilia remains functional in the mutant. The behavioral deficiency is therefore most likely due to a defect in membrane function alone.

Methods of extraction and reactivation of the models were essentially identical to those previously used with *P. caudatum* (*4*). Specimens of *P. aurelia* of Pawn (strain d4-95, carrying a pair of recessive alleles *pw*B *pw*B) and two behavioral wild-type strains (51s and d4-85) (*7*) were washed with a dilute saline solution (*8*), then extracted in the solution which contained 0.005 percent (by volume) octylphenoxy polyethoxyethanol (Triton X-100), 20 m*M* KCl, 10 m*M* tris (hydroxymethyl) aminomethane (tris)–maleate buffer (*p*H was adjusted to 7.0 by NaOH) for 30 minutes at 0° to 1°C. The extracted specimens were washed and equilibrated into a chilled 50 m*M* KCl solution buffered by 10 m*M* tris-maleate-NaOH (*p*H 7.0). The models were left in this equilibration medium for 30 minutes at 0° to 1°C. They were then pipetted into various test solutions which consisted of 50 m*M* KCl solution, buffered to *p*H 7.0, plus test substances at room temperature (17° to 21°C).

Cilia on the models of both Pawn and wild-type specimens were reactivated to beat regularly with metachronal waves in a solution containing ATP and magnesium (*9*). The general orientation of the effective power stroke in reactivated cilia was toward the rear of the specimen when free calcium concentration in the reactivation medium was kept below 10^{-6}*M* (*10*). This caused the models to swim forward like nonextracted live specimens under nonstimulated conditions. When the free calcium concentration was raised above 10^{-6}*M* the orientation of the effective power stroke was reversed, so that the models swam backward as live wild-type specimens do in response to proper stimulus. In a test solution with ATP and calcium (without magnesium) cilia were never reactivated to beat (*11*). In the equilibration medium (no calcium added), these static cilia pointed toward the rear as if resting at the end of the last power strokes. However, when the calcium concentration was above 10^{-6}*M*, these nonbeating cilia swung forward and rested pointing toward the front (Fig. 1). Previous evidence has shown that the anterior shift in the pointing direction in nonbeating cilia is equivalent to the reversal of the power stroke in beating cilia (*4, 12*). Adenosine triphosphate and calcium alone reactivate only the reversal mechanism in the extracted cilia but not the beat. These results are summarized in Table 1. The fact that extracted cilia from Pawn and wild-type specimens reacted to all the test solutions identically reinforces the conclusion that there is no genetic lesion affecting the ciliary apparatus.

The same kinds of tests were performed on several other behavioral mutants of *P. aurelia*, including d4-90 ("Paranoiac"—genotype *Pa Pa*), d4-91 ("Fast-2"— *fna fna*), d4-94 ("Pawn"—*pwA pwA*), d4-96 ("Pawn"—pwB^1 pwB^1), and d4-98 ("Fast-1"—*f f*) (*5*). Triton-extracted models of these mutants were reactivated to swim forward in an ATP-Mg solution and backward in the presence of calcium ions, similar to the models of wild type.

These findings, together with the results from behavioral (*5*) and physiological (*6*) examinations, strongly support the previous proposal that these behavioral mutants are membrane mutants. Our findings also indicate that there are pleiotropic effects by the mutations on neither the mechanism of ciliary beat nor the mechanism of ciliary reversal.

CHING KUNG
Department of Biological Sciences, University of California, Santa Barbara 93106

YUTAKA NAITOH
Department of Zoology, University of California, Los Angeles 90024

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10. Free calcium concentration was controlled by changing the ratio of the concentration of Ca^{2+} to that of ethylenebis(oxyethylenenitrilo)tetraacetic acid in the medium. See H. Portzehl, P. C. Caldwell, J. C. Rüegg, *Biochim. Biophys. Acta* **79**, 581 (1964).
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