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23. The effect of fragment size on thermal stability can be expressed as $\Delta T_m = B/L$ where ΔT_m is the difference in T_m of long DNA fragments and that of fragments of some length, L, and B is a proportionality constant numerically equal to 650 at 0.18*M* Na^+ and 720 at 0.21 *M* Na^+ (*20*). Using this expression for the two criteria in Fig. 1B, I calculate that the minimum length for a stable duplex would be 22 base pairs at 60°C and 0.18*M* Na^+; and 18 base pairs at 50°C and 0.21*M* Na^+.
24. The complexity and genomic content of the repetitive component were calculated as follows: when the labeled DNA was reassociated to C_0t of 1 during the enrichment procedure, 6.2 percent bound to hydroxyapatite. Of this, 30 percent represented the repetitive component and reassociated with a K of 3.5 M^{-1} sec^{-1}. This is equivalent to 1.9 percent of the total DNA (0.3 × 6.2 percent). However, at C_0t of 1, only 78 percent of the repetitive component would have reassociated {100 × [1 − 1/(+ 3.5)]}. Therefore, the actual representation of the repetitive component in the genome is 2.4 percent (1.9/0.78). This value has been rounded to 2 to 3 percent in the text because of the probability of some inaccuracies in the data. The complexity of this component was then calculated as follows: $K_{pure} = K/0.024 = 146\ M^{-1}$ sec^{-1} (*20*). The complexity of the unique component is 2.6×10^7 base pairs (Fig. 1), and the K for this tracer was 0.06 M^{-1} sec^{-1}. Therefore, the complexity of the repeated component is approximately 11,000 base pairs [(0.06/146) (2.6×10^7)].
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Calcium-Induced Contraction of the Rhizoplast of a Quadriflagellate Green Alga

Abstract. *The rhizoplast of* Platymonas subcordiformis *is a contractile organelle. Cyclic contraction and extension are induced by incubating the organism in solutions containing calcium and adenosine triphosphate. Rhizoplast contraction is functionally linked to flagellar activity.*

Striated fibrous roots occur in association with the flagellar and ciliary apparatus of many eukaryotic cells (*1*). Striated roots are thought to be anchoring structures that absorb stress generated by flagellar or ciliary movement (*1*, *2*), or to represent elements of a signal-transmitting network in certain specialized cells (*3*, *4*). The rhizoplast, a striated fibrous root of the green alga *Platymonas subcordiformis* Hazen, is a massive structure that shows a striking resemblance to myofibrils (Fig. 1A), which has prompted speculation by some authors that it may possess muscular or contractile properties (*5*, *6*). In this report, we present evidence that suggests that the rhizoplast of *Platymonas* is a contractile organelle. We describe conditions under which the rhizoplast can be induced to undergo extraordinary contraction and subsequent extension and propose a functional relationship of the rhizoplast to flagellar locomotion.

When *Platymonas* is prepared for electron microscopy, using standard techniques (*7*), the rhizoplast appears extended as shown in Fig. 1A. A rhizoplast is firmly attached (*8*) to a pair of basal bodies at the anterior end of the quadriflagellate cell. A second rhizoplast, out of the plane of section in Fig. 1A, is attached in a similar fashion to the other pair of basal bodies. Both rhizoplasts extend from the flagellar pit region, pass by and are usually appressed to the nucleus, and terminate posteriorly in a ribosome-free, filamentous network adjacent to the plasmalemma.

Contraction is illustrated by comparison of Fig. 1, A to C. Contraction can be induced and sustained through preparation for electron microscopy by the addition of $CaCl_2$ to the medium and fixative at concentrations of 0.1 to 5 m*M*. Under these conditions all rhizoplast profiles demonstrate the organelle in the contracted state. Calcium ions appear to be directly involved in triggering the contractile process (*9*) and have been demonstrated cytochemically in the contracted but not in the extended rhizoplast

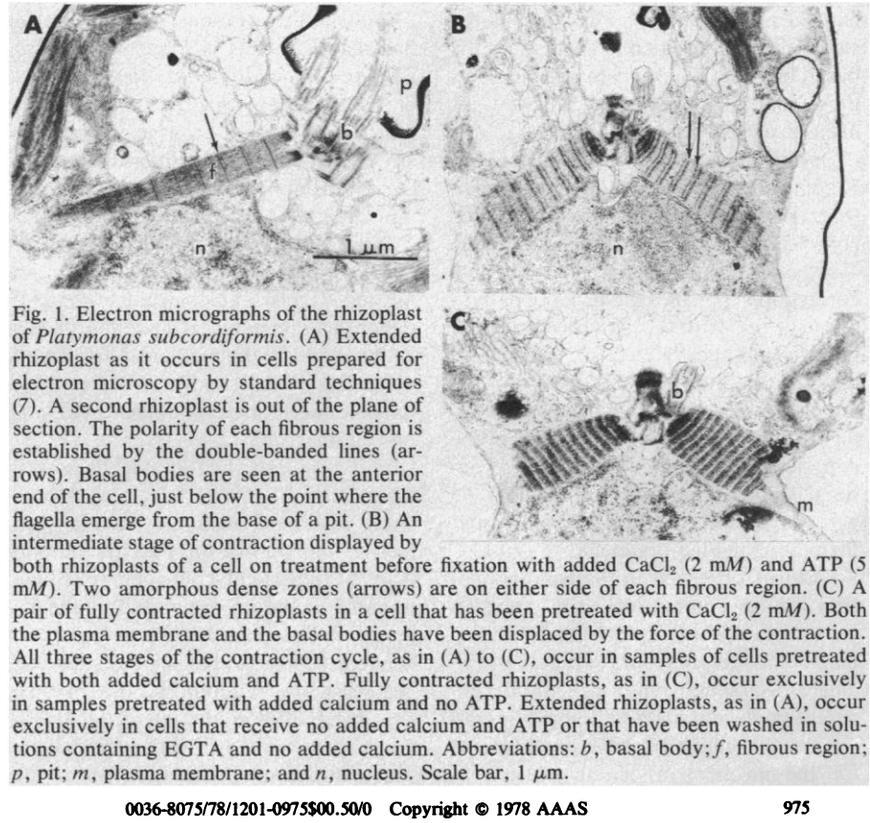

Fig. 1. Electron micrographs of the rhizoplast of *Platymonas subcordiformis*. (A) Extended rhizoplast as it occurs in cells prepared for electron microscopy by standard techniques (*7*). A second rhizoplast is out of the plane of section. The polarity of each fibrous region is established by the double-banded lines (arrows). Basal bodies are seen at the anterior end of the cell, just below the point where the flagella emerge from the base of a pit. (B) An intermediate stage of contraction displayed by both rhizoplasts of a cell on treatment before fixation with added $CaCl_2$ (2 m*M*) and ATP (5 m*M*). Two amorphous dense zones (arrows) are on either side of each fibrous region. (C) A pair of fully contracted rhizoplasts in a cell that has been pretreated with $CaCl_2$ (2 m*M*). Both the plasma membrane and the basal bodies have been displaced by the force of the contraction. All three stages of the contraction cycle, as in (A) to (C), occur in samples of cells pretreated with both added calcium and ATP. Fully contracted rhizoplasts, as in (C), occur exclusively in samples pretreated with added calcium and no ATP. Extended rhizoplasts, as in (A), occur exclusively in cells that receive no added calcium and ATP or that have been washed in solutions containing EGTA and no added calcium. Abbreviations: *b*, basal body; *f*, fibrous region; *p*, pit; *m*, plasma membrane; and *n*, nucleus. Scale bar, 1 μm.

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(*10*). Cytochemical localization of adenosinetriphosphatase activity has been demonstrated in the rhizoplast (*10*) and in other striated roots (*4*, *11*). Cyclic contraction and extension of the rhizoplast can be induced by incubation of the organism in artificial seawater (*7*) with 2 m*M* $CaCl_2$ and 5 m*M* adenosine triphosphate (ATP). This cyclic contraction phenomenon is revealed in living cells by light microscopic observation of repeated pulses of inpocketing along the plasmalemma at the sites of rhizoplast attachment and in fixed cells by comparison of the proportion of electron microscopic images showing rhizoplasts in various states of contraction and extension. Contraction is accompanied by the generation of force sufficiently strong to displace the four basal bodies out of the flagellar pit at the anterior end of the cell and to pull the plasmalemma away from the cell wall at the posterior end of the rhizoplast (Fig. 1, B and C). In addition, the region of the nucleus adjacent to the rhizoplast is distorted. It is doubtful that such an extreme contraction occurs in untreated, normally swimming cells. Rather, a more subtle contraction (*12*) resulting in a tug or pull on the flagellar apparatus seems likely.

The extended rhizoplast of *Platymonas* consists of 10 to 12 fibrous zones, 0.18 μm long, composed of filaments 50 to 70 Å in diameter aligned parallel to the long axis of the organelle. Polarity of the fibrous zones is apparent in the extended organelle and is indicated by the double-banded region (arrow, Fig. 1A) on the side of each fibrous zone away from the basal bodies. Thick filaments such as those found in myofibrils (*13*) have not been observed.

During contraction or extension the banding changes to a complex pattern (Fig. 1B) consisting of two amorphous zones (arrows) on either side of the shortening fibrous region. On complete contraction (Fig. 1C) the banding pattern consists of 10 to 12 amorphous dense bands separated by electron transparent regions. Neither the 50- to 70-Å filaments nor any obvious polarity in structure is apparent in the dense bands of the contracted organelle. The fully contracted rhizoplast is approximately 65 percent shorter and 30 percent wider than the extended organelle.

Changes observed in the banding pattern during contraction and extension are not plainly consistent with the sliding filament model (*14*) of muscle action. Filament disassembly into structurally coherent pools and filament folding or coiling are alternative possible explanations for the mechanism of rhizoplast contraction. Cytochemical evidence suggests a rhizoplast adenosinetriphosphatase concentrated near the double-banded region of the extended organelle (*10*). This adenosinetriphosphatase activity is consistent with both a myosin-like mediated sliding filament model (*13*) and an assembly-disassembly mechanism whereby nucleotide hydrolysis is involved in filament elongation and stability (*15*). Further investigations are needed to elucidate the mechanism of contraction. The information available to date suggests that rhizoplast contraction is (i) triggered by calcium, (ii) cyclic, and (iii) linked to the flagellar apparatus and anchored at the plasmalemma.

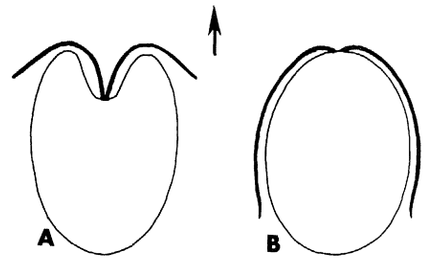

Fig. 2. Schematic representation of the end of a flagellar power stroke for an alga with and without a pit. (A) *Platymonas* profile showing two of the four flagella that emerge from the base of the pit. (B) *Chlamydomonas* profile, without a pit. The maximum degree of bend possible at the proximal end of the flagella is greater in (B) than in (A). The contractile rhizoplast is thought to mechanically aid in the initiation of bend propagation of the return stroke in (A). The arrow indicates the direction of cell movement.

Platymonas, a green alga with anterior flagellation, swims in breaststroke fashion like *Chlamydomonas* (*16*). The power stroke in these algae is an oarlike beat, followed by a bend-propagated recovery stroke that encounters little resistance to the return movement. This type of motion differs from the undulatory waves (*17*) propagated along the flagella of animal spermatozoa that have posteriorly located flagella and forward motion. In either case, bending of cilia and flagella is thought to be the result of ATP-driven, dynein-mediated sliding of adjacent axonemal microtubule doublets relative to one another (*18*).

In *Platymonas* the four flagella arise in a line from the bottom of a deep depression, which places some peculiar geometric constraints on flagellar activity (Fig. 2). In particular, the degree of bend possible at the proximal end of the flagellum is less than that for cells without sunken flagellar bases. Our results suggest that the function of the massive rhizoplast of *Platymonas* is related to these constraints on flagellar motion and that contraction of this organelle may act as a mechanical aid in (i) initiation (*19*) of the flagellar power and recovery strokes, (ii) coordination of the stroke cycle, and (iii) directional control of planar flagellar beat. It is interesting that another alga with a deep depression at the point of flagellar insertion, *Pyramimonas* (*20*), also has a massive rhizoplast. In some motile algae that do not have the physical constraints imposed by a deep apical depression (for instance, *Chlamydomonas*) the large rhizoplast is not present. Rather, the contractile function may be satisfied by the smaller proximal and distal fibers linking the basal bodies (*16*). Fibers of this type resemble miniature rhizoplasts in some respects and are situated appropriately for mechanical stimulation of the basal bodies. Our results do not eliminate a signal-transmitting role (*3*, *4*) as a possible additional function of the rhizoplast.

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8. The rhizoplasts and basal bodies remain attached to one another on isolation.
9. The specificity of calcium for triggering rhizoplast contraction was determined by scoring for inpocketing of the plasma membrane at the sites of rhizoplast attachment after various cation treatments. Calcium was the most effective cation tested; strontium, cobalt, zinc, and manganese (all at 2 m*M*) were 47, 41, 33, and 30 percent as effective, respectively, as calcium, while copper, iron, and lithium did not induce significant contraction. Magnesium was present in all trials as a component of the artificial seawater and was not in itself competent to induce contraction.
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Serotonin Shifts the Phase of the Circadian Rhythm from the *Aplysia* Eye

Abstract. *A putative neurotransmitter, serotonin, may be used to transmit temporal information in the eye of* Aplysia, *because it can shift the phase of the circadian rhythm of spontaneous optic nerve impulses from the eye and the eye contains a significant quantity of serotonin. Serotonin acts either directly on the cell, or cells, containing the circadian pacemaker or on cells electronically coupled to the pacemaker cells.*

Entrainment of circadian pacemakers (CP's) by light-dark (LD) cycles requires processing of the LD information by a photoreceptor and propagation of the information to the pacemaker. Decoding of this information by the pacemaker shifts the phase of the rhythm. The isolated eye of *Aplysia californica* affords an opportunity to study the mechanisms of entrainment. The isolated eye exhibits a circadian rhythm of spontaneous compound action potentials (CAP's) (*1*) which is entrainable by LD cycles in vitro (*2*). Thus a complete entrainment pathway is contained within the eye. Other pathways for entrainment of the eye may exist, because the CP in the eye may be entrained by extraocular photoreceptors (*3*). Also, the circadian pacemaker of one eye may be coupled to the pacemaker in the other eye (*4*).

Some work on the ocular entrainment pathway has been done (*5*). Treatments that inhibited secretion and blocked nerve potentials (tetrodotoxin with high Mg^{2+} and low Ca^{2+} concentrations) did not affect phase-shifting of the CAP rhythm by light pulses. Thus, neither chemical release nor action potentials are required for shifting the rhythm in the eye by light. Treatments which blocked phase-shifting by light (low concentrations of Na^+ and very low concentrations of Ca^{2+}) all caused a reduction of the electroretinogram of the eye by at least 90 percent. This correlation suggests that the translation of light reception by the photoreceptors into a membrane potential change (photoreceptor potential) is an important step in phase-shifting. Further support for the involvement of membrane potential changes comes from the fact that depolarizing stimuli, strophanthidin, and increased concentrations of extracellular K^+ can shift the phase of the rhythm (*6*).

In the course of these entrainment studies a number of putative transmitters were applied to the eye at phase CT 18–24 (*7*). Light pulses significantly advance the rhythm at this phase. None of the transmitter substances produced net phase shifts when applied to the eye at phase CT 18–24. However, a large variation was noted in the effects of serotonin at this phase. We now report that the putative transmitter serotonin shifts the phase of the rhythm of the *Aplysia* eye when it is applied at other phases, and that serotonin acts either directly on the cells containing the CP or on cells electrotonically coupled to the circadian pacemaker cells.

Eyes with optic nerves attached were dissected from *Aplysia* which previously had been entrained to a 12:12 LD cycle. The eyes then were submerged in buffered (Hepes) filtered seawater (BFSW) (15°C) containing penicillin and streptomycin, and were placed in light-tight boxes under constant dark conditions. Optic nerve activity was recorded on a Grass polygraph by means of platinum wire electrodes. Solutions were added and rinsed out of the eyes by polyethylene tubing which was passed into the boxes through light-tight fittings (*8*).

Treatment of the eyes with serotonin ($10^{-5}M$) for 6 hours advanced the circadian rhythm by 3.0 ± 0.4 hours (95 percent confidence interval; $N = 14$) when it was administered at phase CT 05-11 (Fig. 1A); similar treatment administered at phase CT 20-02 delayed the rhythm by 2.5 hours ($N = 2$). In general, treatment with serotonin advanced the rhythm when it was given during the projected day and delayed the rhythm when given during the projected night (Fig. 1B). The transition from delay to advance occurred at approximately projected dawn. Treatment with serotonin produced 4-hour phase shifts of the rhythm in doses as low as $10^{-7}M$ and had no effect on the rhythm in concentrations over $10^{-3}M$. At intermediate concentrations of serotonin (10^{-3} to $10^{-6}M$), 6-hour treatment appeared to saturate the phase-shifting machinery of the eye, because there were no significant differences among the average phase shifts produced by any concentration of serotonin within this range. The failure of serotonin to shift the rhythm at higher concentrations may be due to desensitization of receptors (*9*). Serotonin also decreased the spontaneous CAP activity of the eye at all phases and concentrations tested (2×10^{-3} to $10^{-7}M$).

The effects of serotonin on the spontaneous activity of the eye and the rhythm suggested that this compound might perform a neurotransmitter or neurosecretory function in the eye. Since a prerequisite for demonstrating a transmitter role for serotonin is to show that it is contained in the eye, groups of two to four eyes were assayed for serotonin and other biogenic amines (*10*). The quantities of these substances per eye were 0.25 ± 0.12 ng of serotonin and 0.02 ± 0.01 ng of dopamine ($N = 6$). Since a single eye contains about 5 μg of protein in its retinal cells (*11*) this equals about 50 ng of serotonin per milligram of protein per eye. This is approximately the concentration of serotonin found in the cerebral ganglion of *Aplysia* (*12*) and considerably greater than the concentration found in the serotonin-rich areas of the mammalian brain (*13*). Serotonin and dopamine were also measured in the optic nerve: the concentrations were, per milligram of protein per nerve, 20 ± 11 ng of serotonin and 2 ng of dopamine (14 μg of protein per optic nerve).

The specificity of serotonin in producing the phase shift was examined in several ways. The immediate precursor to serotonin, 5-hydroxytryptophan, did not produce phase shifts (phase CT 05–11, $10^{-5}M$). Dopamine, a putative transmitter found in the eyes, did not produce phase shifts (phase CT 05–11, $10^{-5}M$).

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