anomalous strain observed near Palmdale does not differ significantly from these earlier explanations. The Palmdale strain anomaly could as well be explained by migration of slip on a low-angle thrust as a horizontal detachment plane. Indeed, continued slip on a horizontal detachment plane must ultimately be relieved along a thrust plane, and, if the detachment plane exists, it is likely that it is coupled to the system of thrust faults beneath the Transverse Ranges, perhaps as one great listric fault.

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References

1. J. C. Savage, W. H. Prescott, M. Lisowski, N. King, *Science* **202**, 883 (1978); J. C. Savage and W. H. Prescott, *J. Geophys. Res.* **78**, 6001 (1973).
2. W. H. Prescott and J. C. Savage, *J. Geophys. Res.* **81**, 4901 (1976).
3. J. H. Whitcomb, *Eos Trans. Am. Geophys. Union* **61**, 368 (1980).
4. A. E. Neill, *ibid.* **60**, 810 (1979); oral presentation at the American Geophysical Union meetings, San Francisco, December 1979.
5. T. Mura, *Adv. Mater. Res.* **3**, 1 (1968).
6. D. L. Anderson, *Sci. Am.* **225**, 53 (November 1971).
7. A. H. Lachenbruch and J. H. Sass, in *Proceedings of the Conference on Tectonic Problems of the San Andreas Fault System*, R. L. Kovach and A. Nur, Eds. (Department of Geological Sciences, Stanford University, Stanford, Calif., 1973), p. 192.
8. D. Hadley and H. Kanamori, *Bull. Seismol. Soc. Am.* **68**, 1449 (1978).
9. ———, *Geol. Soc. Am. Bull.* **88**, 1469 (1977).
10. W. Thatcher, *Science* **194**, 691 (1976); J. B. Rundle and W. Thatcher, *Bull. Seismol. Soc. Am.* **70**, 1869 (1980).
11. R. O. Castle, J. N. Alt, J. C. Savage, E. I. Balazs, *Geology* **2**, 61 (1974).

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Cellulose Metabolism by the Flagellate *Trichonympha* from a Termite Is Independent of Endosymbiotic Bacteria

Abstract. *Continuous axenic cultures were established of* Trichonympha sphaerica, *a cellulose-digesting symbiotic protozoon in the gut of a termite. The cultured flagellates harbored no endosymbiotic bacteria and metabolized cellulose to acetate, carbon dioxide, and hydrogen. Thus, the cellulolytic activity of this flagellate is an inherent property and is not dependent on endosymbiotic bacteria.*

Flagellated Protozoa in the order Hypermastigida are mostly large, structurally complex, cellulose-digesting symbiotes in the intestines of termites and the wood-feeding roach *Cryptocercus* (*1-3*). These insects live on a diet of cellulose but possess insufficient cellulolytic activity, thereby rendering them dependent on their flagellates for cellulose digestion (*1-3*). Degradation of cellulose by the flagellates provides the host with usable end products, which have been identified as acetate (plus CO_2 and H_2) for flagellates from the termite *Zootermopsis* (*3*). The origin of cellulolytic activity in the flagellates (*4*) is uncertain (*2, 3*) because they harbor endosymbiotic bacteria (*5*). Pierantoni (*6*) first postulated that bacteria living within termite flagellates are responsible for hydrolyzing cellulose to soluble sugars. This hypothesis, without experimental support (*2, 3, 5*), has nevertheless become quite popular.

Axenic cultivation of cellulose-digesting hypermastigote flagellates would preclude the involvement of living bacteria in flagellate cellulose metabolism. However, continuous cultivation of these flagellates under axenic conditions has not been achieved previously. Trager (*7*) obtained multiplication of *Trichonympha sphaerica* in primary cultures and first subcultures. A mixed population of flagellates, including hypermastigotes, from *Cryptocercus* have been grown in culture for extended periods (*8*). The sensitivity of these flagellates to oxygen (*2, 3*) and their possible complex nutritional requirements (*3*) have probably contributed to their neglect as candidates for detailed study in vitro. Axenic cultivation is now reported for a hypermastigote, *Trichonympha sphaerica*, from the western damp-wood termite *Zootermopsis* (*9*).

The culture technique used was that developed for axenic cultivation of a cellulose-digesting trichomonad flagellate (*10*). The anaerobic medium contained cellulose particles and heat-killed bacteria (*11*). In our study, the intestinal contents of *Zootermopsis* (*12*) were inoculated into the culture medium containing 250 units of penicillin and 250 μg of streptomycin per milliliter, and the inoculated cultures were incubated at 20°C. After 9 weeks, a mixed culture of *Trichonympha sphaerica*, *Trichomitopsis termopsidis*, and intestinal bacteria was obtained. To separate *T. sphaerica* from *T. termopsidis*, we selected 25 cells of the former from primary culture fluid with a micropipette, checked to ensure the absence of trichomonads, and then inoculated them into fresh medium with antibiotics. The flagellates multiplied to several hundred cells after 8 weeks; thereafter, they were subcultured about every 4 weeks. After several further passages, living extracellular bacteria were absent (*13*), and antibiotics were omitted from the medium. Flagellates typically increased tenfold after 4 weeks and reached about 150 cells per milliliter of medium. The cells were actively motile in culture and were filled with cellulose particles (Fig. 1a).

Since endosymbiotic bacteria have been reported in *T. sphaerica* (*14*), the

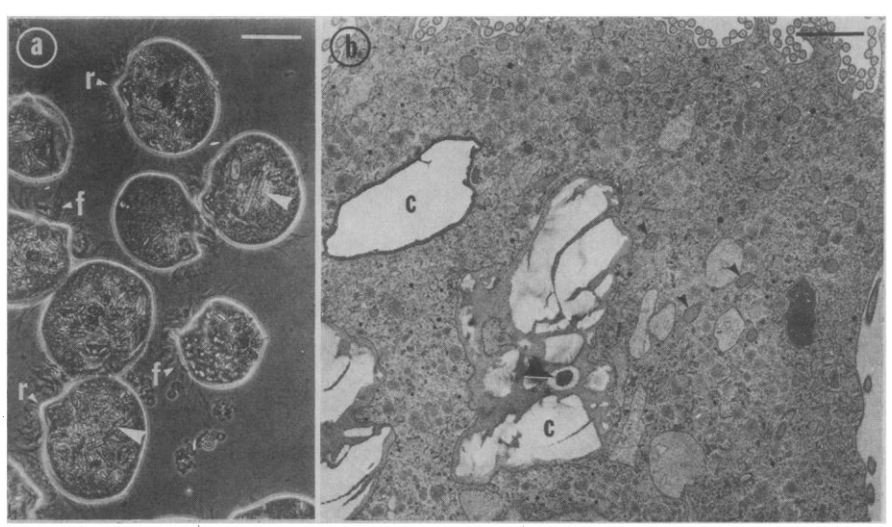

Fig. 1. *Trichonympha sphaerica* from axenic culture. (a) Light micrograph with phase contrast optics of cells fixed with glutaraldehyde. Each cell has an anterior rostrum (*r*), flagella (*f*), and cytoplasm containing many ingested cellulose particles (arrowheads). The bar represents 100 μm. (b) Electron micrograph of part of a cell showing the absence of endosymbiotic bacteria in the cytoplasm. The usual cytoplasmic organelles are present, including microbodies (small arrowheads) and food vacuoles containing cellulose particles (*c*) and a heat-killed food bacterium (large arrowhead). Bar represents 2 μm.

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cultured flagellates were examined by electron microscopy (*10*) to determine whether antibiotic treatment had rendered them endosymbiote-free. No endosymbiotic bacteria were ever observed in these flagellates. They possessed food vacuoles containing cellulose particles and heat-killed food bacteria (Fig. 1b), along with the usual cell organelles present in this and related hypermastigotes (*14, 15*). Since no living bacteria were present in the culture fluid nor within the flagellates, the cultures were axenic. *T. sphaerica* has since been grown in continuous axenic culture for more than 15 months.

Cellulose was required for growth by axenic *T. sphaerica* which suggested that the flagellates possessed enzymes for cellulose metabolism. The end products of cellulose metabolism were determined by growing axenic flagellates in medium containing labeled cellulose (*16*). The results after several weeks of incubation with ^{14}C-labeled cellulose showed that *T. sphaerica* produced CO_2 and acetate, which accounted for about 30 and 58 percent of the label released into the culture fluid, respectively (*17*). Hydrogen was also produced by the flagellates (*18*). These end products are the same as those produced by axenic *Trichomitopsis termopsidis* (*16*) and by *Trichonympha* spp. from *Zootermopsis* (*3*).

These results indicate that *T. sphaerica* is itself capable of cellulose metabolism without the participation of endosymbiotic bacteria. The axenic flagellates metabolized cellulose intracellularly and released acetate, a substrate that would be absorbed from the termite intestine and oxidized (*3*) or used in biosynthesis (*19*). Although numerous symbiotic associations between bacteria and hypermastigote flagellates have been reported (*5, 14*), the assumed role of endosymbiotes in cellulose metabolism by these flagellates should be viewed with skepticism in view of the data presented above.

Hypermastigote flagellates display many interesting characteristics that can now be studied in vitro. They are obligate anaerobes with unusual metabolism (*2, 3*); they ferment cellulose to potentially useful end products, have complex ultrastructures (*14, 15*) and an unusual reproductive process (*8*), and some undergo sexual cycles in response to molting of the host (*20*). These highly evolved organisms should no longer be regarded as interesting but recalcitrant members of the biological world.

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References and Notes

1. L. R. Cleveland, S. R. Hall, E. P. Sanders, J. Collier, *Mem. Am. Acad. Arts Sci.* **17**, 185 (1934).
2. B. M Honigberg, in *Biology of Termites*, K. Krishna and F. M. Weesner, Eds. (Academic Press, New York, 1970), vol. 2, p. 1; ———, in *Chemical Zoology*, G. W. Kidder, Ed. (Academic Press, New York, 1967), vol. 1, p. 695.
3. R. E. Hungate, in *Biochemistry and Physiology of Protozoa*, S. H. Hutner and A. Lwoff, Eds. (Academic Press, New York, 1955), vol. 2, p. 159.
4. ———, *Ecology* **19**, 1 (1938); W. Trager, *Biochem. J.* **26**, 1762 (1932).
5. H. Kirby, in *Protozoa in Biological Research*, G. N. Calkins and F. M. Summers, Eds. (Columbia Univ. Press, New York, 1941), p. 1009; R. A. Bloodgood and T. P. Fitzharris, *Cytobios* **17**, 103 (1976).
6. U. Pierantoni, *Arch. Zool. Ital.* **22**, 135 (1936); ———, *Naturwiss.* **38**, 346 (1951).
7. W. Trager, *Biol. Bull.* **66**, 182 (1934).
8. H. Ritter Jr., S. Inoué, D. Kubai, *J. Cell Biol.* **77**, 638 (1978).
9. The termites *Zootermopsis angusticollis* and *Z. nevadensis* normally harbor seven flagellate species: three hypermastigotes, *Trichonympha campanula*, *T. collaris*, and *T. sphaerica*; the trichomonads *Trichomitopsis termopsidis*, *Tricercomitus termopsidis*, and *Hexamastix termopsidis*; and the oxymonad flagellate *Streblomastix strix*. Only the first four species digest cellulose (*7*). The hypermastigotes are present in greatest abundance and are primarily responsible for cellulose digestion in these termites [L. R. Cleveland, *Biol. Bull.* **48**, 309 (1925)].
10. M. A. Yamin, *J. Protozool.* **25**, 535 (1978). Yeast extract was used at a concentration of 0.5 percent (weight to volume), and cellulose at a concentration of 0.05 percent (weight to volume).
11. These were autoclaved washed rumen bacteria and had no cellulolytic activity [M. A. Yamin and W. Trager, *J. Gen. Microbiol.* **113**, 417 (1979)].
12. These termites harbored *T. sphaerica* as the only hypermastigote present.
13. As determined by lack of bacterial growth in flagellate cultures or in fluid thioglycolate broth (BBL) inoculated with culture fluid.
14. A. V. Grimstone, *J. Biophys. Biochem. Cytol.* **6**, 369 (1959); H. Kirby, *Univ. Calif. Publ. Zool.* **37**, 349 (1932); C. A. Kofoid and O. Swezy, *ibid.* **20**, 99 (1919).
15. A. V. Grimstone and I. R. Gibbons, *Phil. Trans. R. Soc. London, Ser.* B, **250**, 215 (1966); A. Hollande and J. Carruette-Valentin, *Protistologica* **7**, 5 (1971); D. R. Pitelka and C. N. Schooley, *J. Morphol.* **102**, 199 (1958).
16. End products released into the culture fluid were quantified and identified as described [M. A. Yamin, *Appl. Environ. Microbiol.* **39**, 859 (1980)]. Briefly, labeled CO_2 was trapped in phenylethylamine from acidified culture fluid. Labeled acetate was separated and quantified by vacuum distillation and ether extraction; it was identified by thin-layer chromatography and by a substrate-specific reaction with acetate kinase.
17. Average of two determinations on two 7-week-old cultures. Metabolism of labeled cellulose was very slow, but analysis of end products at earlier times gave similar results.
18. Present at a few tenths of a percent in the gas phase above active cultures.
19. J. K. Mauldin, N. M. Rich, D. W. Cook, *Insect Biochem.* **8**, 105 (1978); G. J. Blomquist, R. W. Howard, C. A. McDaniel, *ibid.* **9**, 371 (1979).
20. L. R. Cleveland, *J. Protozool.* **3**, 161 (1956).
21. I thank K.-P. Chang and S. L. Tamm for critically reviewing the manuscript and W. Trager for advice and support of this work.
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Effect of Adenosine 3′,5′-Monophosphate on Neuronal Pacemaker Activity: A Voltage Clamp Analysis

Abstract. *Bursting pacemaker activity in nerve cells can be modified for long periods by synaptic input of short duration. There is evidence that cyclic nucleotides may play a role in these modifications. The predominant effect of elevated levels of adenosine 3′,5′-monophosphate in* Aplysia *neurons was an increased slope conductance to hyperpolarizing pulses, evident in voltage clamp records. A similar increase in slope conductance was seen as one component of maximum strength synaptic stimulation, which is consistent with the idea that cyclic nucleotides are important in the expression of synaptic alteration of bursting pacemaker activity.*

It has been suggested that cyclic nucleotides function as "second messengers" in a number of synaptic events in neural systems. An analysis of the role of cyclic nucleotides in these events has been lacking because of difficulties inherent in obtaining biophysical measurements of membrane responses in complex neural tissue. We used a neuron from the nervous system of the marine mollusk *Aplysia* to perform such an analysis. Cell R15 of *Aplysia* is subject to long-lasting hyperpolarization after brief stimulation of the branchial nerve (*1*). This synaptic response is enhanced in the presence of theophylline (*2*), a phosphodiesterase inhibitor thought to prolong the actions of cyclic nucleotides by preventing their breakdown. Phosphodiesterase inhibitors may have effects on processes other than those related to cyclic nucleotide action. Additional evidence of cyclic nucleotide involvement is the finding that intracellular injection of 5′-guanylyl imidodiphosphate (GMP-PNP), which activates adenylate cyclase, causing increased synthesis of adenosine 3′,5′-monophosphate (cyclic AMP), produces a long-lasting hyperpolarization of cell R15 (*2*).

In our study we use voltage clamp techniques to elucidate the pseudo-steady-state current-voltage (*I-V*) relationship in R15 during nucleotide action. The *I-V* curve induced by this action is compared with that produced by synaptic stimulation. Our analysis serves both to examine the manner in which cyclic AMP exerts its effects and to further test the hypothesis that long-lasting synaptic events are mediated by cyclic nucleotides. The biophysical events must be similar if cyclic nucleotides are mediating the synaptic event.

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