Proc. Natl. Acad. Sci. USA
Vol. 78, No. 11, pp. 7195–7199, November 1981
Neurobiology

Mutational alteration of membrane phospholipid composition and voltage-sensitive ion channel function in *Paramecium*

(lipid mutant/lipid–protein interactions/electrophysiology)

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Communicated by John R. Preer, Jr., July 29, 1981

ABSTRACT **A behavioral mutant of *Paramecium tetraurelia* (*baA*) has been isolated that has an abnormal response when placed in solutions containing Ba^{2+}. This mutant is shown here to have a dramatic alteration of the sphingolipid and phosphonolipid composition of its ciliary membrane. This biochemical defect is present in independently isolated alleles at *baA* locus and segregates in crosses with the behavioral phenotype. Electrophysiologically, the mutation reduces significantly conductance of both voltage-sensitive Ca^{2+} channels and voltage-sensitive K^+ channels. When the mutant is grown in sterol-supplemented medium, its behavior, electrophysiological properties, and lipid composition are hardly distinguishable from wild type grown under similar conditions. This mutant then, provides strong evidence that membrane lipids significantly influence the function of the membrane molecules responsible for the generation of action potentials.**

Many membrane enzymatic and transport functions have been shown to be modulated by the membrane lipid composition (1–3). The membrane proteins responsible for the generation of action potentials are similar to those with enzymatic activity in that their function is influenced by membrane lipids as inferred from indirect studies on the effects of temperature (4), enzyme digestions (5–9), and the application of agents (e.g., local anesthetics or pressure), which are expected to alter the physical properties of membrane lipid (10–13).

The ciliated protozoan *Paramecium tetraurelia* is well suited to the study of the molecular basis of membrane excitability. The cilia of *Paramecium* are covered by a membrane that is continuous with the body surface membrane (14). Membrane depolarization causes these channels to open allowing an influx of Ca^{2+}, which increases transiently the intracellular Ca^{2+} concentration, reversing the direction of the ciliary beat and swimming direction (15, 16). The ciliary membrane is electrophysiologically specialized; it is the exclusive locus of the voltage-sensitive Ca^{2+} channels (17–20). Biochemical analyses of both the protein and lipid components suggest a biochemical specialization for this membrane as well (21–25). Furthermore, over 300 behavioral-mutant lines have been isolated mapping to more than 25 complementation groups (26, 27). The electrophysiological correlates of these behavioral mutations have, in many cases, been determined (28–30). Protein alterations have been found in a few of these mutants (ref. 31; unpublished data). Experiments with temperature changes (ref. 32; unpublished results), local anesthetics (33), and hydrostatic pressure (34) have suggested that lipids also affect ion channel activity in *Paramecium*. In this report we now show that the functions of voltage-sensitive ion channels in the excitable membrane of *Paramecium* appear to be affected by a mutationally induced change in the phospholipid composition of the ciliary membrane. This mutant provides strong evidence that membrane lipids significantly influence the function of membrane proteins responsible for the generation of action potentials.

METHODS

Culture Conditions and Stocks. Cells were grown by routine methods (21, 35). $H_3{}^{32}PO_4$ was added to 0.5 μCi/ml (1 Ci = 3.7×10^{10} becquerels) prior to addition of bacteria to the medium. In some cases stigmasterol (5 mg/liter) was also added to the medium prior to bacteria addition. The strains were: wild type, 51s; *baA-1*, d4-592; *baA-2*, d4-593; *baA-3*, d4-594; *fna*, d4-91; *pwA*, d4-94; *baB*, d5-599 (27).

Cilia Isolation and Lipid Extraction. Cells in late logarithmic growth were harvested, washed extensively, and deciliated; the cilia were separated from bodies as described by Adoutte *et al.* (25). Lipids were extracted as described by Andrews and Nelson (21) except that the initial extract and wash were combined and treated with 2.5 ml of $CHCl_3$ and 2.5 ml of 0.2 M KCl.

Lipid Separation and Quantitation. Phospholipids were separated in two dimensions on analytical (250 μM) thin-layer plates coated with silica gel 60 (EM Laboratories, Elmsford, NY) with a standard solvent system (21). Individual ^{32}P-labeled phospholipids were identified by autoradiography and quantified as described (21).

Electrophysiological Studies. Intracellular recording and voltage clamp were by standard methods (36, 37) with paramecia bathed in 0.5 mM $CaCl_2$/1 mM $Ca(OH)_2$/1 mM citric acid, with the pH adjusted to 7.2 with 1.5 mM Tris at room temperature (free Ca^{2+}, $\approx$0.91 mM).

RESULTS

The *baA* Mutation. *Paramecium* behavioral mutants were isolated after *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine mutagenesis as described by Kung (38). The mutants were selected primarily by their abnormal response to various forms of ionic stimulation. One group of mutants, designated "barium-shy" has an abnormal response when placed in solutions containing Ba^{2+} (ref. 27; unpublished results). Wild-type *Paramecium* normally swim forward in a left-handed helix. When placed in a solution containing Ba^{2+}, the cell undergoes a rapid series of avoiding reactions—i.e., repeated periods of alternating forward and backward swimming, commonly called the "barium dance" (Fig. 1*A*). This behavior reflects repeated depolarizations of the ciliary membrane and influx of Ca^{2+} and Ba^{2+} through the voltage-sensitive Ca^{2+} channels on the ciliary membrane (27). The barium-shy mutants show one long, continuous backward swim-

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Abbreviation: PnE, 1-alkyl-2-acyl-*sn*-glycero-3-(2'-aminoethyl)-phosphonate.

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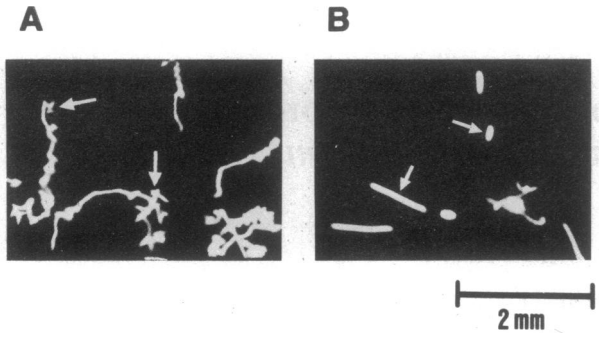

FIG. 1. Behavioral responses to a solution of 8 mM $BaCl_2$/1 mM $CaCl_2$/1 mM Tris·HCl, pH 7.2/10 μM EDTA by wild-type and *baA* cells. Dark-field micrographs were taken at 21°C (38). Continuous lines record the movement of cells during the 5 sec immediately after they are put into the solution. (*A*) Wild-type cells undergo a series of rapid avoiding reactions (arrows) characteristic of the "barium dance." (*B*) *baA* cells show one long, continuous, backward swimming (arrows) in response to the solution.

ming (continuous reversal of ciliary beating) in response to the Ba^{2+} solution (Fig. 1*B*), and eventually die within 1–2 min. The cell seldom "dances"—i.e., resumes short periods of normal forward swimming. Mutants showing this behavioral phenotype map to two different complementation groups designated *baA* and *baB*.

The Phospholipid Composition of Wild-Type and *baA* Ciliary Membranes. At least 13 phosphate-containing lipids have been identified in the ciliary membrane (21, 22). The predominant component is 1-alkyl-2-acyl-*sn*-glycero-3-(2′-aminoethyl)phosphonate (PnE) (Fig. 2 *Upper*) (21, 22). Of particular interest for this study are six sphingolipids, all of which appear to have ethanolamine at their headgroups (Fig. 2 *Upper*, spots A–F). Three of these (spots B, D, and F) are phosphonolipids, and the other three (spots A, C, and E) have the more common phosphodiester linkages at the headgroup. It appears most likely that the three pairs (spots A and B, C and D, and E and F) represent the phospholipid and phosphonolipid derivatives of three long-chain bases, sphingosine, dihydrosphingosine, and phytosphingosine (21).

Mutants in the *baA* locus (*baA*/*baA*, stock d4-592) (Fig. 2 *Bottom*) consistently showed differences from wild type in the relative amounts of the sphingolipids present in the ciliary membrane and also in the amount of PnE. The result of these changes is that the ratio of phosphonosphingolipid to phosphosphingolipid shifted from 1 in wild type to ≈6 in the mutant and that the fraction of total ciliary phospholipid and phosphonolipid that contain ethanolaminephosphonate was 68% in the mutant but only 46% in wild type. No other significant differences between wild-type and *baA* cells was found in any of the other phospholipids of the ciliary membrane or of deciliated bodies (data not shown). The ester-linked fatty acids of *baA* ciliary lipids were not detectably different from those of wild type at the same stage of growth. The sterol composition of *baA* was also indistinguishable from that of wild type, and the sterol-to-phospholipid ratio of *baA* ciliary membrane also was not grossly different from that of wild-type cells (unpublished data). The proteins of *baA* ciliary membrane were analyzed on $NaDodSO_4$/polyacrylamide gels and isoelectric focusing gels (unpublished data) and were indistinguishable from those of wild type.

If this change in ciliary membrane-lipid composition were produced by the genetic lesion at the *baA* locus, independently isolated alleles at this locus should show a similar biochemical defect; in fact, two such alleles (d4-593 and d4-594) did also have very similar alterations in the phospholipid composition of the

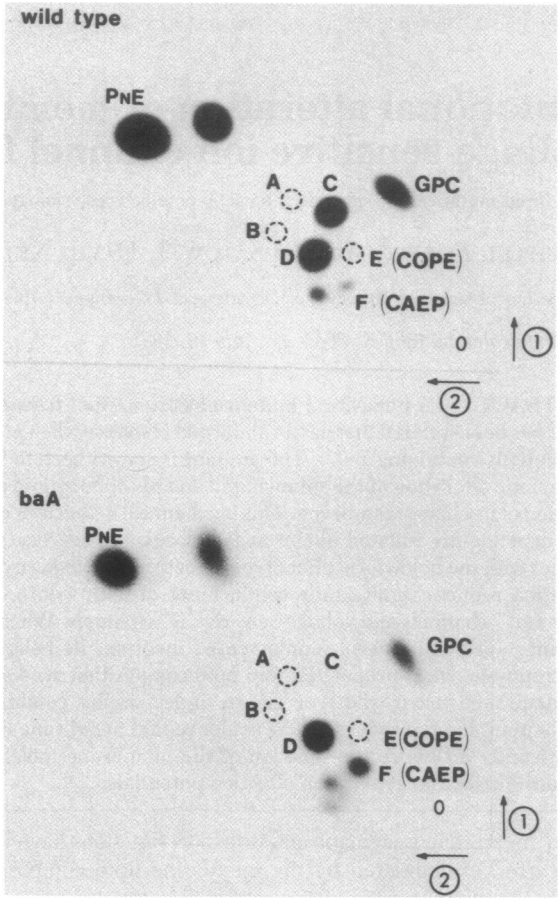

FIG. 2. Two-dimensional thin-layer chromatography of ciliary membrane ^{32}P-labeled lipids. (*Upper*) ^{32}P-Labeled ciliary membrane lipids of wild-type cells. (*Lower*) ^{32}P-Labeled ciliary membrane lipids of *baA* cells (d4-592), with half of the total ^{32}P-labeled lipids as shown in *Upper*. Exposure in both cases was for 70 hr. Spots A, B, and E are minor sphingolipids which appear under a number of different growth conditions (Table 1) (refs. 21 and 22; unpublished data). Their positions are marked here to allow for direct comparison with the previously published studies. GPC, 1-alkyl-2-acyl-*sn*-glycero-3-phosphorylcholine; COPE, ceramide-1-phosphorylethanolamine; CAEP, ceramide-1-(2′-aminoethyl)phosphonate.

ciliary membrane (Table 1). In addition, double mutants could be made between the *baA* mutation and other behavioral mutations. Standard genetic analysis in *P. tetraurelia* involves two meioses [a conjugation and an autogamy (26)], thus making such double mutants F2s of the *baA* parent. The *fast*-2 mutation (*fna*/*fna*, stock d4-91) has defined electrophysiological alterations (30). The *baA*·*fna* double mutant showed the same alteration of ciliary membrane phospholipid and sphingolipid composition as did the *baA* single mutant (Table 1). Behaviorally, these cells expressed both parental phenotypes. Thus, the biochemical alteration segregates in crosses with the barium-shy phenotype produced by this mutation. The *fast*-2 single mutant and a pawn mutant that had a greatly reduced inward Ca^{2+} current (*pwA*/*pwA*, stock d4-94) showed no significant alterations in the ciliary membrane phospholipid composition (Table 1). A mutation in another locus producing the similar barium-shy phenotype (*baB*/*baB*, stock d4-599) also did not show the biochemical change produced by the *baA* mutation (Table 1).

Electrophysiology of *baA* Mutants. Step depolarizations with a voltage clamp can induce a transient inward current carried by Ca^{2+}, followed by an outward current carried by K^+ in *Paramecium* (15, 39, 40). Under optimal conditions (see ref. 37),

Table 1. Phospholipids of *Paramecium* ciliary membranes

Strain	PnE	C	D	E	F	Total Pn	Pnsp(D + F) / Psp(C + E)
+	32.8	12.8	11.8	0.7	1.7	46.4	1.0
+*	42.9	0.3	3.6	1.2	21.8	68.7	16.9
baA-1	41.1	3.3	20.0	0.8	6.4	67.6	6.4
baA-2	38.9	7.3	18.6	1.2	7.5	65.1	3.1
baA-3	37.5	6.0	19.7	0.7	7.8	65.1	4.1
baA·fna	38.7	2.8	21.7	0.7	7.1	67.6	8.2
*baA-1**	42.6	0.7	5.8	1.4	14.1	63.7	9.5
fna	32.6	14.1	14.0	1.2	3.3	50.0	1.1
baB	33.0	12.1	12.4	1.2	2.0	49.2	1.1
pwA	31.9	12.9	13.5	0.9	2.6	48.1	1.2

Results are expressed as percentage of the total ^{32}P-labeled ciliary membrane lipid. All values are the mean of at least three independent determinations. For values greater than 3% of total, the SD was no more than 10% of the mean. PnE, C, D, E, and F refer to the corresponding spots in Fig. 2. +, Wild type; *, cells grown with sterol supplementation; Pn, phosphonolipid; Pnsp, phosphonosphingolipid; Psp, phosphosphingolipid.

the transient Ca^{2+} inward currents induced by step depolarization in wild type and *baA* mutant are shown in Fig. 3 *Top*. The time to peak of the inward currents in wild type [2.1 ± 0.3 msec (n = 7)] and in *baA* mutant [2.4 ± 0.5 msec (n = 6)] are not different. Voltage–current plots show that the peak Ca^{2+} current of *baA* mutant was significantly smaller than that of wild type at all voltage steps above −10 mV ($P < 0.01$ at or above −0.5 mV) (Fig. 4 *A* and *B*).

There are two outward K^+ currents induced by membrane depolarization in *Paramecium*; the voltage-sensitive K^+ current and the Ca^{2+}-induced K^+ current (40, 41). We can eliminate

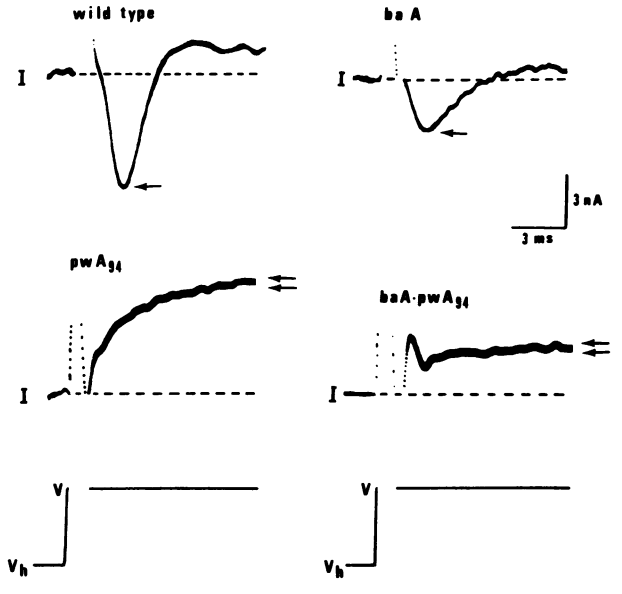

FIG. 3. Current recordings (I) from wild-type and mutant paramecia bathed in the Ca^{2+} solution. The membrane was first held at the resting potential (V_h) (*Bottom*) and was set to a depolarized level (V) with a voltage clamp. The transient inward currents of wild type (*Top Left*) and the *baA* mutant, d4-592 (*Top Right*) were induced by similar voltage steps (V_h = −28 and −27 mV, V = −2 and −0.9 mV in wild type and *baA* mutant, respectively). The peak inward current in *baA* mutant clearly was smaller than that of wild type (single arrows). The early outward current of *baA* mutant was also smaller (double arrows), shown by comparing a pawn mutant, pwA_{94} (*Middle Left*) and the $baA{\cdot}pwA_{94}$ double mutant (*Middle Right*) induced by similar steps (V_h = −32.5 and −31 mV, V = +11 and +12 mV in *pwA* and *baA·pwA*, respectively).

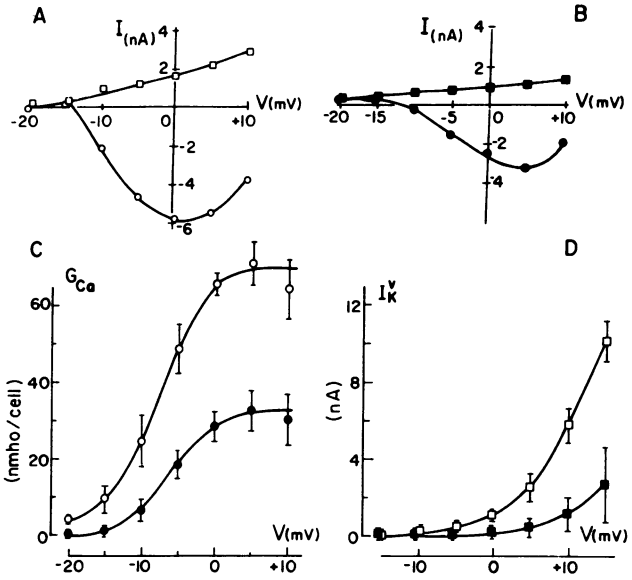

FIG. 4. Membrane currents at different voltages of wild type and *baA* mutant showing reduction of the voltage-sensitive Ca^{2+} current and K^+ current by the *baA* mutation. Membranes were held and then step-depolarized as shown in Fig. 3. (*A*) Current–voltage relationships of wild type (○) shows the inward transients of wild type (mean; n = 5) at different voltages. These are total currents measured near peak times (2.2 msec). To estimate the voltage-sensitive outward currents at 2.2 msec, a pawn mutant, *pwA* (□) (n = 5), was used to discount the Ca^{2+} current and the possible Ca^{2+}-induced K^+ current. (*B*) Current–voltage relationships of *baA* (●) shows the inward currents near peak time (2.3 msec) of *baA* (n = 6) at different voltages. To suppress the Ca^{2+}-related elements and to compare with *A*, *baA·pwA* was used to examine the outward component at 2.3 msec (■) (n = 5). Note that both the total current and the outward component were smaller in paramecia with the *baA* mutation (*B*) than in those without (*A*). (*C*) Ca^{2+} conductances (G_{Ca}) at different voltages calculated from the data shown in *A* and *B*. Note that the conductance of *baA* (●) (mean ± SD; n = 5–7) was ≈ 50% of that of the wild type (○) (n = 5) at all voltages. (*D*) Depolarization-sensitive K^+ currents at different voltages. The currents were measured at 10 msec after the step depolarization to allow K^+ activation. Note that the depolarization-sensitive K^+ currents of *baA·pwA* (■) (n = 6) were clearly smaller than those of *pwA* (□) (n = 5) at all voltages above −5 mV.

the latter from the total current by comparing *baA* mutants to normal cells when both strains also carry a pawn mutation (*pwA/pwA*, from stock d4-94), which blocks the inward Ca^{2+} current and, therefore, also blocks the outward K^+ current induced by raising the internal Ca^{2+} concentrations (40, 41). Thus, comparison of the outward current of a *pwA* mutant and a *baA·pwA* double mutant should reveal any difference through the voltage-sensitive K^+ channel alone (Fig. 3 *Middle*). Voltage–current plots at 10 msec (Fig. 4 *A* and *B*) show that the outward current of *baA·pwA* was significantly smaller than that of *pwA* at all voltages above −5 mV ($P < 0.01$).

The conductance of the inward Ca^{2+} current can be calculated by first subtracting the background current in a pawn mutant (39) and then dividing it by the estimated driving force (40). (Because the peak Ca action potentials are the same in the two strains, the internal Ca^{2+} concentrations are considered to be the same.) The calculated Ca^{2+} conductance of *baA* mutants was smaller than that of the wild type at all voltages above −10 mV (Fig. 4*C*). The maximal Ca^{2+} conductance of this mutant was about 50% of that of the wild type. The voltage at which half-maximal conductance was observed is ≈−8 mV in the wild type and ≈−6 mV in the *baA* mutant, showing no significant change in the voltage sensitivity of the Ca^{2+} channel by the

mutation under these test conditions.

To approximate the voltage-sensitive K^+ current, the leakage current estimated near the holding potential (the leakage conductance is ≈35 nmho per cell in both strains) was further subtracted from the recorded current (41). The K^+ current of *baA* membrane was significantly smaller than that of the normal membrane at all voltages above −5 mV (Fig. 4*D*). The *I–V* relationships in Fig. 4*D* were comparable to the *G–V* (conductance–voltage) relationships because the internal K^+ concentrations of the two strains were the same. The equilibrium potential of K^+ at 4 mM external K^+, as judged by the reversal potential of the inward tail current after step hyperpolarization was −54.8 ± 5.6 mV ($n = 8$) in wild type and −52.7 ± 3.9 mV ($n = 7$) in *baA* mutant.

If K^+ (4 mM) is added to the Ca^{2+} solution, the maximal Ca^{2+} conductance of the wild type becomes smaller (40) (≈40 nmho per cell), and the wild type vs. mutant difference becomes obscure. *baA* cells tested behaviorally in barium solutions containing K^+ (4 mM) also were more similar to wild type. However, the voltage at which half-maximal conductance was seen is about −14 mV in wild type and about −4 mV in *baA* mutant. Thus, under these test conditions, the voltage sensitivity of the Ca^{2+} channels may have been shifted in *baA*. The outward currents in the Ca^{2+}/K^+ solution were not significantly different between the normal and the mutant membranes. The resting potential of the two strains were not different: −31.2 ± 5.2 mV ($n = 6$) in *baA* mutant and −32.0 ± 2.7 mV ($n = 7$) in wild type in the Ca^{2+} solution; −31.0 ± 3.5 mV ($n = 6$) in *baA* and −32.6 ± 4.2 mV ($n = 8$) in wild type in the Ca^{2+}/K^+ solution (K^+, 4 mM).

Effects of Sterol Supplementation. The sphingolipid composition of the wild-type ciliary membrane was altered dramatically when the culture medium was supplemented with sterol (stigmasterol, 5 mg/liter) (Table 1). Sterol supplementation increased the amount of sterol present in the ciliary membrane by up to 10-fold (unpublished data). The results of sterol-induced change were a large increase (from 1 to 17) in the ratio of phosphonosphingolipids to phosphosphingolipids and a significant increase (from 46% to 68%) in the proportion of total phospholipids and phosphonolipids that contain ethanolamine phosphonate (Table 1). The phospholipid compositions reported in Table 1 for sterol-supplemented cells were similar to those previously reported (21, 22). *baA* cells grown in sterol-supplemented Cerophyl only altered their sphingolipid composition slightly. In *baA* mutant, the addition of sterol raised the phosphonosphingolipid-to-phosphosphingolipid ratio less than 2-fold. The effect of these changes was to make the composition of *baA* mutant and wild-type cells very similar in the sterol-supplemented medium (Table 1). If the altered phospholipid composition causes the electrophysiological defects of *baA*, then the growth of *baA* in sterol-supplemented medium might be expected to suppress them. Therefore, the electric parameters described above were reexamined by comparing either wild type and *baA* or *pwA* and *baA·pwA* grown in the presence of added sterol. The peak inward current and the current–voltage relationships of cells carrying the *baA* mutation were more similar to, if not identical with, control cells.

DISCUSSION

We have found an alteration in the sphingolipid and phosphonolipid composition of the excitable membrane of *Paramecium* that is associated with mutationally induced behavioral and electrophysiological defects. The alteration is found in three alleles at this locus but not in any of ≈20 other mutants electrophysiologically or behaviorally defective that map at other loci. In addition, the lipid alteration segregates with the *baA* gene in crosses. When the mutant is grown in the stigmasterol-supplemented medium, its behavior, electrophysiological properties, and lipid composition are hardly distinguishable from those of wild-type cells grown in the same medium. The *baA* mutation is clearly pleiotropic; it affects at least two ion channels that function independently of one another.

The altered lipid composition in *baA* is not due to an absolute lack of some biosynthetic enzyme because the mutant contains some of each of the lipids found in wild type; it is only their relative proportions that are altered in the mutant. The mutant may be defective in the environmental regulation of phosphonolipid synthesis or turnover. Wild-type cells "adapt" to low concentrations of sterol in the growth medium by altering the proportion of phospholipids in their ciliary membranes. Thus, changes in the ratios of the phospholipids alone do not inevitably affect behavior. However, the *baA* mutant barely changes its lipid composition in response to this change in its environment. Alternatively, the lesion in *baA* may be in the uptake or metabolism of sterols or in the kind of fatty acid associated with the ciliary lipids, which indirectly forces a compensating change in phospholipid composition. Because the sterol and fatty-acid composition of *baA* and wild-type ciliary membranes are not grossly different, small changes in these components would have to induce these compensating changes in phospholipid composition.

The *baA* mutation produces a complex set of alterations in at least two of the voltage-sensitive ion channels. The observations in any particular case depend, however, on the composition of the test solution or the growth condition. The results suggest that the *baA* mutation does not affect directly the channel proteins themselves because essentially wild-type activity in *baA* cells can be observed when the phospholipid composition is changed. The results suggest but do not prove that the lipid alteration directly causes changes in the properties of two different voltage-sensitive ion channels. There are at least three different but not mutually exclusive ways in which lipids might influence ion channels: (*i*) by acting as specific cofactors to stabilize or activate the ion channel protein; (*ii*) by providing a microenvironment, the viscosity (fluidity) of which influences membrane proteins; or (*iii*) by contributing to the surface charge or ion-binding capacity of the ciliary membrane. The first possibility seems least likely; sphingolipids are present in cilia in large molar excess over *all* membrane proteins. Second, fluidity of the lipids in which membrane proteins act has been shown in many cases (42–45) to alter their function. Because the sphingolipid composition of the ciliary membrane changes in response to conditions known to alter the fluidity of membranes (46, 47), these lipids may play a crucial role in determining the fluidity. Several recent findings single out sphingolipids as having important effects on membrane fluidity (48–50). In addition, sphingolipids in general appear to have a unique relationship with sterols, and it has been suggested that these two lipids form a complex (51–55). As shown here, the *baA* mutation alters the sphingolipid composition, which may then alter the fluidity of the ciliary membrane under specific environmental conditions and, thus, the functioning of the ion channels. In this case, the channels may respond directly to the fluidity of the membrane or to some change in the mixing of sterol in the membrane induced by the altered lipid composition. Lastly, the head groups of phosphonolipids and phospholipids are likely to have different cation-binding properties. It is possible that a subtle difference in bound surface-charge pattern, which influences excitability (15, 56), could account for the electrophysiological differences between wild type and mutant.

In short, it appears that membrane lipid composition affects the ion channels of *Paramecium*, but we are not yet able to offer a simple explanation for the effect. We have in this study focused on phospholipids, but it will clearly be important to determine if subtle compensating changes in fatty acid or sterol composition accompany the phospholipid changes in *baA* mutant. The analysis of this mutation can also be extended by making double mutants of *baA* and other mutations. Novel phenotypes arising in such double mutants can suggest interesting interactions.

This work was funded by U.S. Public Health Service Grant GM22714 to C.K. and D.N., National Science Foundation Grant BNS79-18554 to C.K., and National Institutes of Health Postdoctoral Fellowship GM06491 to M.F.

1. Fourcans, B. & Jain, M. K. (1974) in *Advances in Lipid Research*, eds. Paoletti, R. & Kritchevsky, D. (Academic, New York), pp. 147–226.
2. Sanderman, H. (1978) *Biochim. Biophys. Acta* **515**, 207–237.
3. Barenholz, Y. & Thompson, T. E. (1980) *Biochim. Biophys. Acta* **604**, 129–158.
4. Romey, G., Chickeporticle, R. & Lazdunski, M. (1980) *Biochim. Biophys. Acta* **602**, 610–625.
5. Knickelbein, T. G. & Rosenberg, P. (1980) *J. Toxicol.* **18**, 71–79.
6. Tobias, D. J. (1955) *J. Cell Comp. Physiol.* **46**, 183–189.
7. Albuquerque, E. X. & Thesleff, S. (1967) *J. Physiol. (London)* **190**, 123–132.
8. Tasaki, I. & Takenaka, T. (1964) *Proc. Natl. Acad. Sci. USA* **52**, 804–810.
9. Cook, A. M., Low, E. & Ishijimi, M. (1972) *Nature (London) New Biol.* **239**, 150–152.
10. Lee, A. G. (1976) *Nature (London)* **262**, 545–548.
11. Papahadjopoulos, D. (1972) *Biochim. Biophys. Acta* **265**, 169–186.
12. Wann, K. T. & MacDonald, A. G. (1980) *Comp. Biochem. Physiol.* **66**, 1–12.
13. Famaya, H., Ueda, I., Moore, P. S. & Eyring, H. (1979) *Biochim. Biophys. Acta* **550**, 131–137.
14. Dute, R. & Kung, C. (1978) *J. Cell Biol.* **79**, 451–464.
15. Eckert, R. & Brehm, P. (1979) *Annu. Rev. Biophys. Bioeng.* **8**, 353–374.
16. Eckert, R., Naitoh, Y. & Machemer, H. (1976) in *Calcium in Biological Systems*, ed. Duncan, C. J. (Cambridge Univ. Press, Cambridge, England), pp. 233–255.
17. Dunlap, K. (1977) *J. Physiol. (London)* **271**, 119–131.
18. Ogura, A. & Takahashi, K. (1976) *Nature (London)* **264**, 170–172.
19. Machemer, H. & Ogura, A. (1979) *J. Physiol. (London)* **296**, 49–57.
20. Brehm, P. & Eckert, R. (1978) *Science* **202**, 1203–1205.
21. Andrews, D. & Nelson, D. L. (1979) *Biochim. Biophys. Acta* **550**, 174–187.
22. Rhoades, D. E. & Kaneshiro, E. S. (1979) *J. Protozool.* **26**, 329–339.
23. Kaneshiro, E. S., Beischel, L. S., Merkel, S. J. & Rhoades, D. E. (1979) *J. Protozool.* **26**, 147–158.
24. Hansma, H. G. & Kung, C. K. (1975) *Biochem. J.* **152**, 523–528.
25. Adoutte, A., Ramanathan, R., Lewis, R. M., Dute, R. R., Ling, K. Y., Kung, C. & Nelson, D. L. (1980) *J. Cell Biol.* **84**, 717–738.
26. Sonneborn, T. M. (1974) in *Handbook of Genetics* (Plenum, New York), pp. 469–594.
27. Nelson, D. L. & Kung, C. (1978) in *Taxis and Behavior*, ed. Hazelbauer, G. L. (Chapman and Hall, London), pp. 77–100.
28. Satow, Y. & Kung, C. (1976) *J. Membr. Biol.* **28**, 277–294.
29. Satow, Y. & Kung, C. (1976) *J. Exp. Biol.* **65**, 51–63.
30. Satow, Y. & Kung, C. (1976) *J. Neurobiol.* **7**, 325–338.
31. Merkel, S. J., Kaneshiro, E. S. & Gruenstein, E. I. (1981) *J. Cell Biol.* **89**, 206–215.
32. Hennessey, T. & Nelson, D. L. (1979) *J. Gen. Microbiol.* **112**, 337–348.
33. Browning, J. L. & Nelson, D. L. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 452–457.
34. Otter, T. & Salmon, E. D. (1979) *Science* **206**, 358–360.
35. Sonneborn, T. M. (1950) *J. Exp. Zool.* **113**, 87–148.
36. Naitoh, Y. & Eckert, R. (1972) in *Experiments in Physiology and Biochemistry*, ed. Kierkut, G. A. (Academic, London), Vol. 5, pp. 17–38.
37. Satow, Y. & Kung, C. (1979) *J. Exp. Biol.* **78**, 149–161.
38. Kung, C. (1971) *Genetics* **69**, 29–45.
39. Oertel, D., Schein, S. J. & Kung, C. (1977) *Nature (London)* **286**, 120–124.
40. Satow, Y. & Kung, C. (1980) *J. Exp. Biol.* **84**, 57–71.
41. Satow, Y. & Kung, C. (1980) *J. Exp. Biol.* **88**, 293–303.
42. Kimelberg, H. K. (1977) in *Dynamic Aspects of Cell Surface Organization*, eds. Poste, G. & Nicolson, G. L. (North-Holland, New York), pp. 205–221.
43. Lands, W. E. M. (1980) *Biochem. Soc. Trans.* **8**, 25–37.
44. Chapman, D. & Hoffman, W. (1980) *Biochem. Soc. Trans.* **8**, 32–40.
45. Metcalfe, J. C., Bennett, J. P., Hesbeth, T. R., Houslay, M. D., Smith, G. A. & Warren, G. B. (1976) in *The Structural Basis of Membrane Function*, eds. Hatefi, Y. & Djavadi-Ohaniance, L. (Academic, New York), pp. 57–67.
46. Shinitzky, M. & Henkart, P. (1979) *Int. Rev. Cytol.* **60**, 121–139.
47. Thompson, G. A. & Nozawa, Y. (1977) *Biochim. Biophys. Acta* **472**, 55–92.
48. Gilmore, R., Cohn, N. & Glaser, M. (1979) *Biochemistry* **18**, 1042–1049.
49. Gilmore, R., Cohn, N. & Glaser, M. (1979) *Biochemistry* **18**, 1050–1056.
50. Schmidt, C. F., Barenholz, Y., Huang, C., & Thompson, T. E. (1978) *Nature (London)* **271**, 775–776.
51. Cullis, P. R. & Hope, M. M. (1980) *Biochim. Biophys. Acta* **597**, 533–542.
52. Estep, T. N., Mountcastle, D. B., Barenholtz, Y., Biltonen, R. L. & Thompson, T. E. (1979) *Biochemistry* **18**, 2112–2117.
53. Patton, S. (1970) *J. Theor. Biol.* **29**, 489–497.
54. Demel, R. A., Jansen, J. W., Van Dyck, P. W. & Van Deenen, L. L. (1977) *Biochim. Biophys. Acta* **465**, 1–10.
55. Snyder, B. & Freire, E. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 4055–4059.
56. Satow, Y. & Kung, C. (1981) *J. Membr. Biol.* **59**, 179–190.