Isolation and Characterization of Paramecium Mutants Defective in Their Response to Magnesium

Robin R. Preston* and Ching Kung*,‡

**Laboratory of Molecular Biology and ‡Department of Genetics, University of Wisconsin-Madison, Madison, Wisconsin 53706*

Manuscript received December 6, 1993
Accepted for publication March 7, 1994

ABSTRACT

Four mutant strains of *Paramecium tetraurelia* with a reduced ability to respond behaviorally to Mg^{2+} have been isolated. Voltage-clamp analyses showed that their Mg^{2+} insensitivity is associated with a reduced Ca^{2+}-dependent Mg^{2+} current. The four mutants, which have been dubbed "eccentric," result from recessive mutations in two unlinked loci, *xntA* and *xntB*. Further analysis of *xntA*^{1} showed it to be unlinked to any of the behavioral mutants of *P. tetraurelia* described previously, but it is allelic to d4-521, a "K^+-resistant" strain, and d4-596, a "Ba^{2+}-shy" mutant. The varied pleiotropic effects of *xntA*^{1}, which include increased resistance to Ni^{2+} and Zn^{2+} poisoning, suggest that the locus encodes a central regulator of cell function in Paramecium.

ACTION potentials are such simple forms that it is easy to forget that they represent an exquisitely choreographed sequence of underlying ionic events. Attempts to dissect these events rapidly reveals their true complexity, however, in terms of both the variety of ion fluxes involved, and also in the number of membrane components that regulate these fluxes. One particularly powerful and successful means of unraveling the complexities of membrane excitation involves genetic mutation. Although such mutations may occur naturally [for example, naturally occurring mutations in a gene that encodes a chloride channel causes cystic fibrosis in humans (DALEMANS *et al.* 1991; DRUMM *et al.* 1991), whereas a base substitution in a human sodium-channel gene causes hyperkalaemic periodic paralysis (ROJAS *et al.* 1991)], it is more practical to induce genetic lesions artificially and then search for phenotypes that may indicate excitation anomalies. The success of this approach is readily apparent from attention now focused on the Drosophilia *shaker* gene, which encodes a voltage-dependent K channel (PAPAZIAN *et al.*, 1987). In addition, systematic searches for channel mutants in the ciliated protozoan, Paramecium, have revealed that calmodulin is essential for normal Ca^{2+}-dependent K channel and Ca^{2+}-dependent Na channel function in this organism (KINK *et al.* 1990; SAIMI and LING 1990; see PRESTON *et al.* 1991).

Recently, Paramecium was discovered to express a Mg^{2+}-specific conductance that is elicited upon either step depolarization or hyperpolarization under voltage clamp (PRESTON 1990). This current is unusually interesting, not only because it is the first of its kind and for its possible role in shaping membrane excitability in Paramecium, but also for the possible repercussions of this Mg^{2+} influx on cell activity. For many years, cells were believed to contain Mg^{2+} concentrations of the order of 30 mM, far in excess of the optimum ($\leq$1 mM) required by the many Mg^{2+}-dependent intracellular enzymes and regulatory proteins. However, with the advent of ion-specific fluorescent dyes and electrodes has come the realization that intracellular *free* Mg^{2+} concentrations ($[Mg^{2+}]_i$) generally rest at around 0.3–0.7 mM (see ALVAREZ-LEEFMANS *et al.* 1987). This discovery was quite surprising, for it suggests that even small changes in $[Mg^{2+}]_i$ could have major effects on Mg^{2+}-dependent intracellular activity. The full significance of this finding becomes apparent when one considers that cells expend energy using Mg^{2+}-specific transporters to maintain $[Mg^{2+}]_i$ at this low level against a strong electrochemical gradient (see FLATMAN 1984, 1991; BEYENBACH 1990), and that these transporters are under hormonal control in several clonal cell lines (MAGUIRE 1984; GRUBBS 1990). Although the evidence linking changes in $[Mg^{2+}]_i$ with a specific cellular response remains equivocal at present, there seems little doubt that Mg^{2+} will eventually be recognized to be a critical determinant of cell function (GRUBBS and MAGUIRE 1987; WHITE and HARTZELL 1989; ROMANI and SCARPA 1992). Thus, since activating I_{Mg} must inevitably change $[Mg^{2+}]_i$ (even if transiently), this current could have a role beyond simply changing membrane potential in Paramecium.

In attempts to better understand the molecular basis and possible functions of I_{Mg}, we initiated a search for mutants that might be defective in this conductance. The electrophysiological characteristics of one such mutant are described elsewhere (PRESTON and KUNG 1994); here we present a behavioral and genetic analysis of this and other newly isolated "eccentric" mutants of *Paramecium tetraurelia*.

MATERIALS AND METHODS

Stocks and culture conditions: The present studies were conducted using *P. tetraurelia*, stock 51s, and the following

mutants derived from this stock: d4-90 paranoiac A (*PaA/PaA*) (Van Houten *et al.* 1977), d4-91 fast-2 (cam^{11}/cam^{11}, formerly *fna/fna*) (Kung 1971; Kink *et al.* 1990) d4-94 pawn A (*pwA/pwA*) (Kung 1971), d4-95 pawn B (*pwB/pwB*) (Kung 1971), d4-97 fast-1A (*fA/fA*) (Kung 1971), d4-98 fast-1B (*fB/fB*) (Kung 1971), d4-149 paranoiac (cam^3/cam^3, formerly fna^P/fna^P) (Van Houten *et al.* 1977; Kink *et al.*, 1990), d4-150 paranoiac C (*PaC/PaC*) (Van Houten *et al.* 1977), d4-152 TEA-insensitive A (*teaA/teaA*) (Chang and Kung 1976), d4-521 K^+-resistant (Shusterman *et al.* 1978), d4-565 paranoiac D (*PaD/PaD*) (Van Houten *et al.* 1977), d4-580 pawn C (*pwC/pwC*) (Satow *et al.* 1974), d4-596 Ba^{2+}-shy (Shusterman 1981), d4-619 pantophobiac B (*pntB/pntB*) (Hinrichsen *et al.* 1985), d4-622 pantophobiac A (cam^1/cam^1, formerly *pntA/pntA*) (Hinrichsen *et al.* 1985; Kink *et al.* 1990), d4-623 dancer (Dn^1/Dn^1) (Hinrichsen *et al.* 1984), d4-644 k-shy A (ksA^1/ksA^1) (Evans and Nelson 1989), d4-645 k-shy B (*ksB/ksB*) (Evans and Nelson 1989), d4-646 paranoiac F (*PaF/PaF*) (Saimi and Kung 1987), d4-647 restless (*rst/rst*) (Richard *et al.* 1985), d4-700 eccentric A ($xntA^1/xntA^1$), d4-701 eccentric A ($xntA^2/xntA^2$), d4-703 eccentric A ($xntA^3/xntA^3$) and d4-704 eccentric B (*xntB/xntB*). All eccentric mutants were isolated during the present studies. A trichocyst non-discharge mutation (*nd6/nd6*) (Lefort-Tran *et al.* 1981) was used as a genetic marker in all of the crosses described below. All stocks were raised at 28° on a wheat grass infusion supplemented with 5 mg/liter stigmasterol and inoculated with *Enterobacter aerogenes*, as described by Sonneborn (1970).

Solutions: All solutions contained 1 mM $CaCl_2$, 1 mM HEPES, 0.01 mM EDTA, pH 7.2. Chloride salts of barium, cobalt, magnesium, manganese, nickel, potassium, sodium or zinc were added to this solution as required and at the concentrations stated. Solutions containing magnesium were additionally supplemented with 10 mM tetraethylammonium (TEA^+) chloride. "Resting solution" contained 4 mM KCl.

Mutagenesis: Mutations were induced in *nd6* cells using *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, as described by Kung (1971). After 1-hr exposure to mutagen, cell populations were separated into 8 or 16 groups, starved to induce homozygosity via autogamy, and then allowed to undergo 6–8 fissions prior to screening.

Mutant selection: Mutants lacking a behavioral response to Mg^{2+} were selected using a galvanotactic technique, similar to that described by Hinrichsen *et al.* (1984). A sample of 100 ml of mutagenized cells in late logarithmic growth phase was concentrated by centrifugation and washed twice in resting solution. After 10-min adaptation in this solution, the cells were loaded into the holding compartment of a galvanotactic trough. After a 5-min rest, voltage was applied across the length of the trough (40 V, administered in the form of 50-msec pulses at a frequency of 4 Hz). This caused the cells to orient with their anterior poles directed toward the cathode. Lifting the "starting gates" then exposed the cells to 5 mM Mg^{2+}, causing any individuals that were capable of responding normally to Mg^{2+} to swim backward, and hence be drawn toward the anode. Conversely, motile individuals that were unable to respond to Mg^{2+} swam forward toward the cathode, from whence they were isolated for subsequent single-cell cloning and analysis.

Behavioral tests: Five to 10 cells from late logarithmic phase cultures were transferred to resting solution and left undisturbed for 15 min. Individual cells were then selected with a micropipette and ejected forcibly into a test solution containing either 5 mM Mg^{2+} and 10 mM TEA^+, 6 mM Ba^{2+}, 10 mM Na^+ or 30 mM K^+. These solutions were designed to elicit backward swimming in the wild type, the duration of which was recorded with a stopwatch. Such tests were repeated using a minimum of 10 cells. All behavioral tests were carried out at room temperature (23 ± 2°).

Electrophysiology: The techniques used to record the membrane currents of Paramecium under two-electrode voltage clamp have been described elsewhere (Preston *et al.* 1992). Mg^{2+} currents were elicited from cells bathed in 5 mM Mg^{2+} and 10 mM TEA^+, using glass capillary microelectrodes filled with 4 M CsCl, and were analyzed using methods described by Preston (1990).

Divalent cation resistance: Twenty cells suspended in ≈10 μl of culture fluid were transferred to a glass depression containing 850 μl of test solution. At stated times, each depression was examined using a dissecting microscope and its contents scored for cell survival as follows (arbitrary units): fast forward swimming = 5, forward swimming = 4, slow forward swimming = 3, immobile = 2, swollen = 1, blistered (dead) = 0. Intermediate stages were scored accordingly; for example, if a depression contained 10 slowly swimming cells and 10 immobile cells, it was assigned a score of 2.5. iC_2 values, the concentration of divalent required to immobilize cells (a score of 2) following a 2-hr exposure, were determined from plots of survival score against cation concentration.

Genetic analyses: Standard techniques were used to establish genetic relationships between different strains of Paramecium (Sonneborn 1970). Two homozygous strains were crossed by conjugation to yield heterozygous F_1 progeny. Autogamy was then induced in the F_1, producing an F_2 generation that was again homozygous at all loci. Thus, a cross between the two unlinked loci, $a/a \times +/+$, yields an exconjugant F_1 of $a/+$ and a 1:1 ratio of a/a and $+/+$ in the F_2, with no heterozygotes. Twenty mating pairs were isolated during each cross. Exconjugant cells were separated and allowed at least 8 fissions prior to testing the behavior of the F_1. Clones derived from four of these pairs were carried through autogamy. The genotype of all putative double mutants described here was confirmed in backcrosses to the wild type. The *nd6* mutation was used as a genetic marker in all crosses to ensure that cross-fertilization had occurred between two mating cells and that autogamy had been induced successfully in the F_1. *nd6* has no effect on the behavioral or electrophysiological phenotypes of *P. tetraurelia* (R. R. Preston and Y. Saimi, unpublished). Mutageneses were carried out on a population of *nd6* cells; this genetic marker was removed from mutants of interest (by crossing to the wild type) before they were characterized further.

RESULTS

Behavioral responses to Mg^{2+}: Wild-type paramecia typically respond to being transferred to solutions containing 5 mM Mg^{2+} by turning repeatedly. These turns reflect transient membrane depolarizations as Ca^{2+} and Mg^{2+} enter the cells via a depolarization-activated Ca^{2+} current (I_{Ca}) and a Ca^{2+}-dependent Mg^{2+} current (I_{Mg}). If membrane repolarization is prevented by adding 10 mM TEA^+ (a common K^+-channel inhibitor) to the bath, this repeated turning behavior is transformed into a continuous backward swimming episode that can last up to 20 sec. The backward swimming usually terminates in a characteristic "Mg^{2+} dance," comprising forward swimming for about 1 sec, followed by a slow backward swimming episode that returns the cell to its location prior to forward swimming, and then an approximate 40° reorientation of swimming direction. This sequence is repeated many times over a period of up to a minute, so

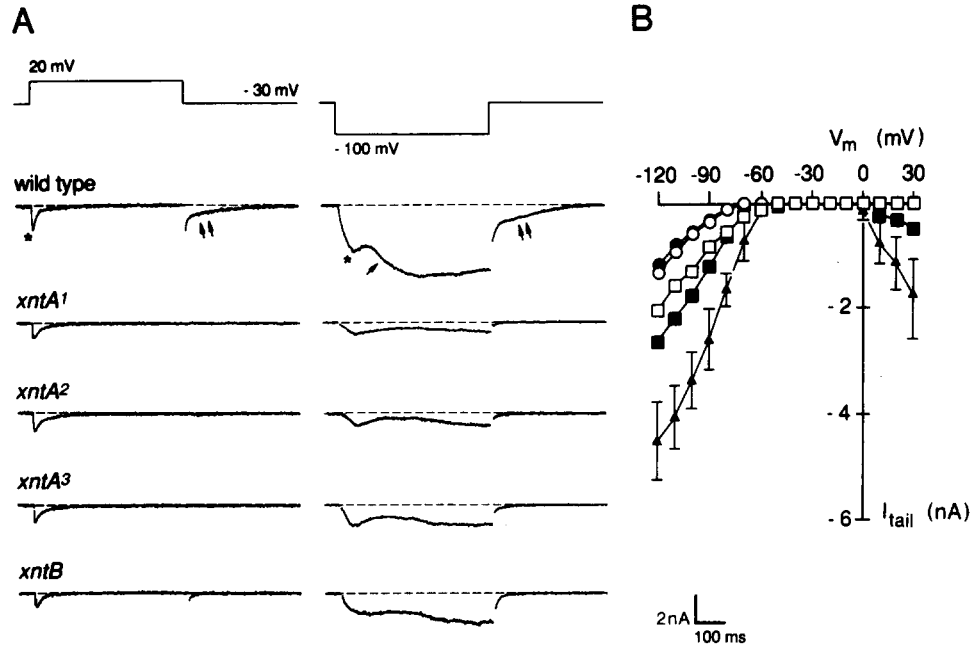

FIGURE 1.—Suppression of magnesium current by eccentric mutations. (*A*) Membrane currents were elicited under a two-electrode voltage clamp from cells bathed in 5 mM Mg^{2+}. Traces shown at left were evoked by 500-msec depolarizations to 20 mV from −30 mV, whereas traces on the right were triggered by hyperpolarization to −100 mV (500 msec). These steps elicit first either depolarization- or hyperpolarization-activated Ca^{2+} currents (indicated by the asterisks in the wild-type traces), and then the slower Mg^{2+} current. The tail currents observed following a return to holding potential (double arrows) are relatively pure Mg^{2+} current. (*B*) Mg^{2+} tail-current amplitudes (I_{tail}) plotted as a function of membrane potential (V_m). Points are mean ± SD determinations from 14 wild-type (triangles), 5 eccentric A^1 (open circles), 13 eccentric A^2 (filled circles), 8 eccentric A^3 (open squares) or 5 eccentric B (filled squares) cells.

that the cell traces a starburst pattern in time and space. The Mg^{2+} dance eventually degenerates into slow forward swimming interupted at frequent but irregular intervals by slow cell turning.

Four classes of cells defective in this Mg^{2+} response were obtained following three separate mutageneses. The first class comprised "pawns" and "atalantas," mutants that were unable to reverse their cilia (KUNG 1971; HINRICHSEN and KUNG 1984) and were thus discarded. The second class included two individuals that were rendered insensitive to Mg^{2+} and all other cations by preincubation in resting solution. These phenotypes have not been described previously, but apparently result from dominant, single-gene mutations (R. R. PRESTON, unpublished results). Since these phenotypes are nonspecific, they were not studied further here. The final two classes express an "eccentric" phenotype, and are the subjects of the present studies. Eccentrics are readily distinguished from the wild type by their "resting" behavior in culture fluid: typically their forward swimming is punctuated at frequent intervals by periods of rapid and abrupt backward swimming. *xntA*^{1}, *xntA*^{2}, and *xntA*^{3} cells all responded to being transferred from resting solution to 5 mM Mg^{2+}/10 mM TEA^+ with either brief (<0.5 sec) backward swimming, with forward swimming or, more commonly, with a series of 5–6 rapid turns. Comparing the behavior of the wild type and *xntA* after an hour's incubation in 5 mM Mg^{2+} showed that whereas the wild-type cells were confined to the bottom of the test well by repeated, slow turning episodes, the mutants were still swimming fast forward. *xntB* cells exhibit an intermediate phenotype, typically swimming backward for 4–7 sec upon transfer to 5 mM Mg^{2+}/10 mM TEA^+, followed by forward swimming at rates that approximate those in the absence of Mg^{2+}.

Ca^{2+}-dependent Mg^{2+} current: As mentioned above, wild-type paramecia swim backward when transferred to 5 mM Mg^{2+}/10 mM TEA^+ because Mg^{2+} enters the cell via I_{Mg} and causes membrane depolarization. Thus, one might expect eccentric's impaired ability to respond behaviorally to Mg^{2+} to reflect a reduction in the magnitude of I_{Mg}. This notion was confirmed during a recent electrophysiological characterization of *xntA*^{1} (PRESTON and KUNG 1994), in which I_{Mg} was found to be missing at physiological membrane potentials. *xntA*^{2}, *xntA*^{3} and *xntB* were examined under whole-cell voltage clamp for a similar current defect. The results are shown in Figure 1. Depolarization of wild-type paramecia in 5 mM Mg^{2+}/10 mM TEA^+ (Figure 1A, upper left) elicits first a transient Ca^{2+} current (asterisk), and then a slower activating Mg^{2+} current. This current also deactivates (turns off after the voltage step) slowly, so that returning to −30 mV following the step depolarization elicits a prominent Mg^{2+} tail current (double arrow). I_{Mg} is also activated upon hyperpolarization of the wild type (Figure 1A, upper right), apparent as an inward current that

TABLE 1

Crosses between eccentric and a trichocyst non-discharge mutant

Cross[a]	F_1 phenotype[b]	F_2 phenotypes +:*xnt*:*nd6*:*xnt*;*nd6*	P^c
$xntA^1 \times nd6$	Wild type	43:37:46:36	>0.7
$xntA^2 \times nd6$	Wild type	35:31:29:30	>0.9
$xntA^3 \times nd6$	Wild type	17:16:17:21	>0.9
$xntB \times nd6$	Wild type	19:16:29:19	>0.2

[a] The recessive trichocyst non-discharge mutation (*nd6*) was used routinely as a marker to ensure that cross-fertilization had occurred during conjugation. *nd6* does not affect the swimming behavior of Paramecium.

[b] F_1 and F_2 generations were tested for discharge competence using a saturated solution of picric acid. Behavioral phenotypes were determined from the duration of backward swimming induced by transferring individual cells to 5 mM Mg^{2+}. At least 5 cells from each ex-conjugant clone from 4 mating pairs were tested for Mg^{2+}-behavior.

[c] Chi-squared values were calculated on the basis of an expected segregation ratio of 1:1:1:1 in each cross, with three degrees of freedom.

develops slowly during the voltage step (arrow), and again as a slowly decaying tail current upon returning to holding potential (double arrow). The amplitudes of these tail currents as a function of membrane potential are shown in Figure 1B. Depolarization of $xntA^1$, $xntA^2$ and $xntA^3$ fails to elicit a Mg^{2+} current: note the lack of a Mg^{2+} tail current upon returning to holding potential (Figure 1, A and B). Hyperpolarization to −100 mV and below does elicit I_{Mg} in these three mutants, but the magnitude of this current is reduced greatly compared with wild-type values (Figure 1B). As might be expected from its behavioral response to Mg^{2+}, *xntB* expresses Mg^{2+}-current upon both de- and hyperpolarization (Figure 1A), but in amounts that are reduced by *ca.* 50% of wild-type values (Figure 1B).

Genetics of eccentric: The four eccentric strains were crossed to *nd6*, a recessive trichocyst non-discharge strain of Paramecium (Lefort-Tran *et al.* 1981). The F_1 progeny of all four crosses showed wild-type behavior in 5 mM Mg^{2+} (Table 1). Inducing autogamy in these heterozygous F_1 cells yielded four classes of F_2 in a 1:1:1:1 ratio with respect to Mg^{2+} behavior and ability to discharge trichocysts (Table 1), indicating that *xntA* and *xntB* are recessive, single-site mutations, and that they are unlinked to *nd6*.

$xntA^2$, $xntA^3$ and *xntB* were next crossed to $xntA^1$ to determine genetic relationships between the four mutations. The $xntA^1 \times xntA^2$ and $xntA^1 \times xntA^3$ crosses both yielded F_1 progeny that failed to respond behaviorally to Mg^{2+} (Table 2). Following autogamy, testing the F_2s for their response to 5 mM Mg^{2+} showed all to behave like their eccentric parents: none exhibited a wild-type response (Table 2). The inclusion of the trichocyst marker in the crosses ensured that cross-fertilization had occurred. These results suggest that the three mutations form a single complementation group, *xntA*. In contrast, the F_1s resulting from a cross between $xntA^1$ and *xntB* showed wild-type responses to Mg^{2+}. Further, testing the F_2s in 5 mM Mg^{2+} showed a 1:1:2 ratio of +:*xntB*:*xntA* (Table 2), suggesting that *xntB* forms a second complementation group that is unlinked to *xntA*.

Heavy metal resistance: We were interested in determining whether eccentric is allelic to any other behavioral mutants of *P. tetraurelia*. Also, since $xntA^1$ has pleiotropic effects on several different ion currents in Paramecium (Preston and Kung 1994), we were interested in knowing how this would affect the responses of existing mutants to various ionic solutions. First, we sought a non-behavioral means of discerning the eccentric phenotype, so as to be able to score progeny from crosses of eccentric to epistatic gene mutants. The conductance pathway that supports I_{Mg} is permeable to several heavy metals that are highly toxic to Paramecium, including Co^{2+}, Mn^{2+}, Ni^{2+} and Zn^{2+} (Preston 1990; R. R. Preston, unpublished). Thus, we reasoned that a mutant lacking I_{Mg} might be resistant to these cations. We first examined the sensitivity of wild-type and eccentric paramecia to Ni^{2+}. Ni^{2+} immobilizes and subsequently kills Paramecium in a concentration-dependent manner (Figure 2, filled symbols): the concentration required to immobilize the wild type by 2 hr (iC_2) is 28 μM (Table 3). Ni^{2+} also kills eccentric, but the mutant's sensitivity is reduced by a factor of 10 compared with the wild type (iC_2 = 278 μM; Figure 2, open symbols; Table 3). By 24 hr, all wild-type cells exposed to ≥20 μM Ni^{2+} had died, whereas the mutant remained unaffected by Ni^{2+} concentrations of ≤50 μM. This disparity provided us with a simple, non-behavioral means of testing for the eccentric trait. The F_2 progeny of an $nd6 \times xntA^1$ cross were tested both for their ability to respond behaviorally to 5 mM Mg^{2+} and for their ability to survive a 24-hr exposure to 20 μM Ni^{2+}: the two phenotypes cosegregate. Eccentric is also resistant to Zn^{2+} and to a lesser extent to Co^{2+} and Mn^{2+} (Table 3).

We also examined eccentric B and various other behavioral mutants for Ni^{2+} resistance. These studies were conducted approximately 6 months after compiling the data listed in Table 3, and showed an even greater difference between iC_2 values for the wild type and eccentric A (Table 4). They also showed eccentric B and Ca-channel mutant pawn B (see below) to be weakly Ni^{2+}-resistant (Table 4). Paranoiac F, a mutant that overresponds to Na^+ solutions, is also Ni^{2+}-resistant (Table 4).

Relationships between eccentric and other Paramecium behavioral mutants: The behavior of eccentric in solutions designed specifically to accentuate the activity of the various ionic conductances of Paramecium was assessed and compared with wild-type responses to the same solutions (Table 6). Wild-type cells respond to Ba^{2+} with a series of avoidance reactions ("dancing"), reflecting passage of this cation through the depolarization-activated Ca channel. Eccentric reacts to Ba^{2+} by swimming backward continuously for many tens of seconds,

TABLE 2

Crosses between strains expressing eccentric behavior

Cross[a]	F_1 phenotype[b]	F_2 phenotypes		P^c
		+:*nd6*	+:*xntA*	
$xntA^1 \times xntA^2$	Eccentric	90:93	0:183	
$xntA^1 \times xntA^3$	Eccentric	51:55	0:116	
$xntA^1 \times K^+$-resistant	Eccentric	54:46	0:100	
$xntA^1 \times Ba^{2+}$-shy	Eccentric	49:60	0:109	
			+:*xntB*:*xntA*	
$xntA^1 \times xntB$	Wild type	80:62	38:36:72	>0.9

[a] The trichocyst non-discharge mutation (*nd6*) was used as a genetic marker to ensure that cross-fertilization had taken place. This marker segregates in an approximate 1:1 ratio among the F_2 in each cross.

[b] Behavioral phenotypes of F_1 and F_2 cells were assessed by testing their ability to swim backward continuously in 5 mM Mg^{2+}, and for their ability to survive a 24-hr exposure to 20 μM Ni^{2+}.

[c] Chi-squared value was calculated on the basis of an expected segregation ratio of 1:1:2, with 2 d.f.

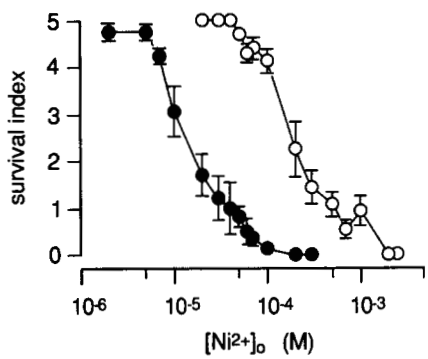

FIGURE 2.—Survival of wild-type and eccentric A mutant cells in nickel. Twenty wild-type or eccentric mutant cells were incubated for 2 hr in various concentrations of $NiCl_2$, then examined visually. The cells were scored for survival (survival index) as described in the text: a value of 5 indicates no difference from control cells that had not been exposed to Ni^{2+}, whereas a value of 0 indicates death. Points are means ± SD of 7 determinations, filled circles indicate the wild type, open circles indicate mutant values.

consistent with the observed slowing of Ca^{2+} current (I_{Ca}) inactivation under voltage clamp (see DISCUSSION). Both strains respond to 10 mM Na^+ with *ca.* 3 sec of backward swimming (Table 6). The wild type recovers slowly from Na^+ stimulation, typically whirling for several seconds before resuming forward swimming, and even then, forward swimming in Na^+ is slow and interrupted frequently by repeated turns. By contrast, eccentric pulls out of the backward swimming very rapidly, resuming fast forward swimming immediately with only occasional turns. K^+ (30 mM) causes eccentric to swim backward for almost twice as long as the wild type, again consistent with the *eccentric* mutation's pleiotropic effects on I_{Ca} (see DISCUSSION).

Eccentric was crossed first to pawn A and pawn B, two strains with recessive mutations (KUNG 1971) that prevent expression of I_{Ca} (OERTEL *et al.* 1977; SATOW and KUNG 1980) and thereby render cells incapable of swimming backward in response to Ba^{2+}, Mg^{2+}, Na^+, or K^+ (Table 6). Eccentric was also crossed to pawn C, a temperature-sensitive mutant that behaves as a leaky pawn when grown at room temperature (23°), but as a full pawn following 24 hr of growth at 35° (SATOW *et al.* 1974; Table 6). The F_1 heterozygotes from all three crosses were wild type (Table 5) with respect to their behavior in 5 mM Mg^{2+}/10 mM TEA^+. The F_2 segregated in a 1:1:1:1 ratio of wild type:pawn: eccentric:double mutant, as judged by their ability to swim backward and their Ni^{2+} resistance (Table 5). The double mutants, *pwA;xntA*^{1} and *pwB;xntA*^{1}, both behaved as pawns (Table 6), while the *pwC;xntA*^{1} double mutant acted as a very leaky pawn at 23°, but as a full pawn following growth at a restrictive temperature (Table 6).

Eccentric was next crossed to dancer, a semidominant mutation (HINRICHSEN *et al.* 1984) that interferes with I_{Ca} inactivation and thereby greatly prolongs the duration of backward swimming in depolarizing solutions (HINRICHSEN and SAIMI 1984; Table 6). The F_1 responded to 5 mM Mg^{2+} with prolonged backward swimming that was statistically indistinguishable from that of the dancer parent. Na^+ (10 mM) caused the F_1 to

TABLE 3

Heavy metal resistance of wild-type and eccentric cells

Metal ion	iC_2[a]		*n*
	Wild type	Eccentric	
Ni^{2+}	28 ± 11	278 ± 102 μM	12
Zn^{2+}	23 ± 15	120 ± 45 μM	5
Co^{2+}	1.7 ± 0.9	6.8 ± 2.4 mM	5
Mn^{2+}	4.7 ± 0.6	10.6 ± 2.9 mM	6

[a] iC_2 indicates the concentration of divalent cation required to immobilize cells following a 2-hr exposure. Data are means ± SD of *n* determinations.

TABLE 4

Ni^{2+} resistance of various behavioral mutants

Cell strain	iC_2 (μM)[a]	*n*
Wild type	17 ± 4	5
Eccentric A	583 ± 126	3
Eccentric B	24 ± 3	6
Pawn B	33 ± 10	4
Paranoiac F	61 ± 21	6

[a] iC_2 indicates the concentration of divalent cation required to immobilize cells following a 2-hr exposure. Data are means ± SD of *n* determinations.

TABLE 5

Crosses between eccentric and various mutant strains

Cross[a]	F_1 phenotype[b]	F_2 segregation[c]		P^d
		+:*nd6*	+:*pwA*:*xntA*^{1}:*pwA*;*xntA*^{1}	
pwA × *xntA*^{1}	Wild type	72:66	42:35:30:31	>0.5
		+:*nd6*	+:*pwB*:*xntA*^{1}:*pwB*;*xntA*^{1}	
pwB × *xntA*^{1}	Wild type	80:77	49:35:36:37	>0.3
		+:*nd6*	+:*pwC*:*xntA*^{1}:*pwC*;*xntA*^{1}	
pwC × *xntA*^{1}	Wild type	95:97	48:50:51:43	>0.8
		+:*nd6*	+:*Dn*^{1}:*xntA*^{1}:*Dn*^{1};*xntA*^{1}	
Dn^1 × *xntA*^{1}	Dancer	65:53	24:31:33:30	>0.5
		+:*nd6*	+:*teaA*:*xntA*^{1}:*teaA*;*xntA*^{1}	
teaA × *xntA*^{1}	Wild type	84:90	42:52:34:46	>0.7
		+:*nd6*	+:*cam*^{1}:*xntA*^{1}:*cam*^{1};*xntA*^{1}	
cam^1 × *xntA*^{1}	Wild type	82:62	35:34:37:38	>0.9
		+:*nd6*	+:*pntB*:*xntA*^{1}:*pntB*;*xntA*^{1}	
pntB × *xntA*^{1}	Wild type	65:48	33:24:33:23	>0.1
		+:*nd6*	+:*rst*:*xntA*^{1}:*rst*;*xntA*^{1}	
rst × *xntA*^{1}	Wild type	58:53	30:31:26:24	>0.7
		+:*nd6*	+:*cam*^{11}:*xntA*^{1}:*cam*^{11};*xntA*^{1}	
cam^{11} × *xntA*^{1}	Wild type	88:73	38:38:46:39	>0.7
		+:*nd6*	+:*cam*^{3}:*xntA*^{1}:*cam*^{3};*xntA*^{1}	
cam^3 × *xntA*^{1}	Wild type	106:103	58:59:46:46	>0.3
		+:*nd6*	+:*PaA*:*xntA*^{1}:*PaA*;*xntA*^{1}	
PaA × *xntA*^{1}	Partial paranoiac	60:45	34:29:22:20	>0.1
		+:*nd6*	+:*PaC*:*xntA*^{1}:*PaC*;*xntA*^{1}	
PaC × *xntA*^{1}	Partial paranoiac	70:68	31:42:37:30	>0.3
		+:*nd6*	+:*PaD*:*xntA*^{1}:*PaD*;*xntA*^{1}	
PaD × *xntA*^{1}	Partial paranoiac	94:85	42:51:44:42	>0.7
		+:*nd6*	+:*PaF*:*xntA*^{1}:*PaF*;*xntA*^{1}	
PaF × *xntA*^{1}	Wild type	66:64	31:32:33:34	>0.9
		+:*nd6*	+:*ksA*^{1}:*xntA*^{1}:*ksA*^{1};*xntA*^{1}	
ksA^1 × *xntA*^{1}	Wild type	90:78	47:43:41:37	>0.7
		+:*nd6*	+:*ksB*:*xntA*^{1}:*ksB*;*xntA*^{1}	
ksB × *xntA*^{1}	Wild type	75:67	38:34:39:31	>0.7
		+:*nd6*	+:*fA*:*xntA*^{1}:*fA*;*xntA*^{1}	
fA × *xntA*^{1}	Wild type	55:47	30:25:23:24	>0.7
		+:*nd6*	+:*fB*:*xntA*^{1}:*fB*;*xntA*^{1}	
fB × *xntA*^{1}	Wild type	52:54	30:33:25:18	>0.1

[a] The recessive trichocyst non-discharge mutation (*nd6*) was used to ensure the success of cross-fertilization during conjugation. This marker segregated from its wild-type allele among the F_2 in an approximate 1:1 ratio in each of the crosses shown.

[b] F_1 phenotypes were scored by testing their behavior in 5 mM Mg^{2+} and, in many crosses, their behavior in 10 mM Na^+. At least 8 F_1 cells from each pair were tested, and at least 4 pairs were examined during each cross.

[c] F_2 phenotypes were typically scored on the basis of Ni^{2+} resistance, Mg^{2+} behavior and their behavior in at least one additional test solution.

[d] Chi-squared values were calculated assuming segregation ratios of 1:1:1:1, with 3 d.f.

swim backward for *ca.* 20 sec, *i.e.*, like a partial dancer. Autogamy yielded F_2 cells that segregated in a 1:1:1:1 ratio of wild type:dancer:eccentric:double mutant (Table 5). The *Dn;xntA*^{1} double mutant presented a phenotype that was different from either parent. Its response to Ba^{2+} was increased significantly compared with either dancer or eccentric, whereas its responses to Na^+ and K^+ were intermediate (Table 6).

Eccentric was next crossed to four mutants that affect K^+ currents in Paramecium. TEA-insensitive A (*teaA*) responds to most depolarizing stimuli with whirling instead of backward swimming (Table 6), due to an overactive Ca^{2+}-dependent K^+ current (Hennessey and Kung 1987). The F_1 from a *teaA* × *xntA*^{1} cross were wild type and the F_2 segregated in a 1:1:1:1 ratio of wild type:TEA-insensitive:eccentric:double mutant. The double mutant behaved like TEA-insensitive A in Na^+ and K^+, but like eccentric in Mg^{2+} and Ba^{2+} (Table 6). Pantophobiac A (*cam*^{1}) is a calmodulin-gene mutant that lacks Ca^{2+}-dependent K^+ currents (Saimi *et al.* 1983; Preston *et al.* 1990b). This deficit prevents normal recovery from depolarization, and hence the cell swims backward for excessive periods in all of our test solutions (Table 6). Crossing pantophobiac to eccentric yielded F_1 cells that were wild type and F_2 cells that segregated in a 1:1:1:1 ratio of wild type:pantophobiac:eccentric:double mutant. The double mutant behaved much as the pantophobiac parent: interestingly, though Ni^{2+}-resistant, the double mutant responded to Mg^{2+} with several seconds of backward swimming (Table 6). Pantophobiac B (*pntB*) resembles pantophobiac A in its tendency to overreact to most ionic stimuli, but the underlying electrophysiological defects are less severe (R. R. Preston, unpublished data). A cross between pantophobiac B and eccentric yielded F_1 cells that were wild type in terms of behavior, while the F_2 segregated in a 1:1:1:1 ratio of wild type:pantophobiac: eccentric:double mutant. The double mutant overreacted to all of our test solutions

TABLE 6

Behavior of wild type and mutants in various ionic solutions

Cell line	Solution[a]				
	Ni^{2+}	Mg^{2+}	Ba^{2+}	Na^+	K^+
Wild type	+	CCR (10)	AR	CCR (3)	CCR (17)
xntA^1	+++	AR	CCR (55)	CCR (3)	CCR (30)
pwA	+	FS	FS	FS	Whirl
pwB	++	FS	FS	FS	FS
pwC (23°)	+	CCR (3)	CCR (19)	CCR (2)	CCR (8)
pwC (35°)	+	Whirl	FS	Whirl	Whirl
Dn	+	CCR (25)	Spin (62)	CCR (106)	CCR (105)
teaA	+	Whirl	AR	Whirl	CCR (30)
cam^1	+	CCR (80)	CCR (45)	CCR (95)	CCR (27)
pntB	+	CCR (89)	CCR (28)	CCR (62)	CCR (25)
rst	+	CCR (13)	AR	FS	CCR (49)
cam^{11}	+	CCR (12)	CCR (24)	FS	CCR (16)
cam^3	+	CCR (25)	AR	CCR (40)	CCR (13)
PaA	+	CCR (12)	AR	CCR (70)	CCR (17)
PaC	+	CCR (8)	AR	CCR (48)	CCR (34)
PaD	+	CCR (10)	AR	CCR (18)	CCR (16)
PaF	++	CCR (47)	CCR (9)	CCR (64)	CCR (23)
ksA	+	CCR (7)	Whirl	CCR (11)	CCR (26)
ksB	+	CCR (7)	AR	CCR (9)	CCR (28)
fA	+	CCR (21)	AR	CCR (14)	CCR (11)
fB	+	CCR (21)	AR	CCR (7)	CCR (12)
pwA;xntA^1	+++	FS	Whirl	FS	FS
pwB;xntA^1	+++	FS	FS	FS	FS
pwC:xntA^1 (23°)	+++	Whirl	CCR (65)	AR	CCR (18)
pwC;xntA^1 (35°)	+++	Whirl	FS	Whirl	Whirl
Dn;xntA^1	+++	AR	Spin (77)	CCR (53)	CCR (62)
teaA;xntA^1	+++	Whirl	CCR (60)	whirl	CCR (32)
cam^1*;xntA*^{1}	+++	CCR (2)	CCR (82)	CCR (31)	CCR (39)
pntB;xntA^1	+++	AR	CCR (74)	CCR (45)	CCR (35)
rst;xntA^1	+++	Whirl	AR	FS	CCR (36)
cam^{11}*;xntA*^{1}	+++	AR	CCR (>200)	FS	CCR (43)
cam^3*;xntA*^{1}	+++	AR	CCR (84)	CCR (41)	CCR (34)
PaA;xntA^1	+++	AR	CCR (35)	CCR (77)	CCR (42)
PaC;xntA^1	+++	AR	AR	CCR (56)	CCR (46)
PaD;xntA^1	+++	AR	CCR (60)	CCR (8)	CCR (37)
PaF;xntA^1	+++	CCR (2)	CCR (88)	CCR (40)	CCR (45)
ksA;xntA^1	+++	Whirl	Whirl	CCR (2)	CCR (54)
ksB;xntA^1	+++	Whirl	AR	Whirl	CCR (61)
fA;xntA^1	+++	AR	CCR (81)	CCR (4)	CCR (22)
fB;xntA^1	+++	AR	CCR (63)	CCR (3)	CCR (21)

AR, avoiding reactions or brief turning behavior. Each turn lasts <1 sec; CCR, continuous ciliary reversal, leading to backward swimming for the times indicated in parentheses (mean values, given in seconds); FS, forward swimming; Whirl, propellor-like rotation about the posterior pole of the cell; Spin, rotation about the cell's longitudinal axis. Each entry indicates results of tests carried out on a minimum of 10 cells.

[a] Solutions were 20 μM Ni^{2+} and 1 mM K^+, 5 mM Mg^{2+} and 10 mM TEA^+, 6 mM Ba^{2+}, 10 mM Na^+, 30 mM K^+. Ni^{2+}-resistance was scored as follows: +++, 100% (of 20 cells) survival after 24-hr exposure; ++, 100% survival after 8 hr, up to 50% survival after 24 hr; + 0% survival at 8 hr.

except Mg^{2+}, which prompted avoidance reactions only (Table 6). Finally, eccentric was crossed to restless (*rst*), a mutant in which the Ca^{2+}-dependent K^+ current elicited upon hyperpolarization activates abnormally fast (PRESTON *et al.* 1990a). Again, the F_1 cells from this cross were wild type, whereas the F_2 segregated in a 1:1:1:1 ratio of wild type:restless:eccentric:double mutant. The double mutant failed to respond to Mg^{2+} with backward swimming, but otherwise behaved like the restless parent. The double mutant was also tested for its ability to survive a 24-hr incubation in low K^+ (0.1 mM, data not shown): like the restless parent (RICHARD *et al.* 1985), the double mutant succumbs rapidly to K^+ withdrawal.

Eccentric was next crossed to a collection of mutants that respond abnormally to Na^+. fast-2 (*cam*^{11}) lacks a Ca^{2+}-dependent Na^+ current (SAIMI 1986) and thus fails to swim backward in 10 mM Na^+ (Table 6). Conversely, paranoiac P (*cam*^{3}) enhances this current and thus causes prolonged backward swimming in response to Na^+ stimulation (KINK *et al.* 1990). Both phenotypes result from mutation of the calmodulin gene, as does that of pantophobiac above (KINK *et al.* 1990). Crossing *xntA*^{1} to pantophobiac had already shown these two genes to be unlinked (Table 5), but we were interested to know how *cam*^{11}*;xntA*^{1} and *cam*^{3}*;xntA*^{1} double mutants would respond behaviorally in various ionic solutions. The results of these crosses are shown in Tables 5 and 6. The *cam*^{11}*;xntA*^{1} double mutant failed to swim backward in Mg^{2+} or Na^+, as expected. Unexpectedly, however, this double mutant showed inordinate sensitivity to Ba^{2+} stimulation. The *cam*^{3}*;xntA*^{1} construct was

Mg^{2+}-insensitive, like the eccentric parent, but exhibited prolonged backward swimming in Na^+, a characteristic of paranoiac P. Mutations in four additional genes yield a paranoiac (excessive Na^+ response) phenotype; paranoiac A (*PaA*), paranoiac C (*PaC*), paranoiac D (*PaD*) and paranoiac F (*PaF*). Eccentric was crossed to *PaA*, *PaC* and to *PaD*, with essentially similar results in each case. F_1 cells from all three crosses were weak paranoiacs with respect to their Na^+ behavior, consistent with previous studies showing *PaA*, *PaC* and *PaD* to be semidominant mutations (VAN HOUTEN *et al.* 1977). All three crosses yielded F_2 cells that segregated in a 1:1:1:1 ratio of wild type:paranoiac:eccentric:double mutant (Table 5). The three double mutants all failed to respond behaviorally to Mg^{2+} but, like their paranoiac parents, swam backwards for tens of seconds upon being transferred to 10 mM Na^+ (Table 6). Eccentric was also crossed to *PaF*, with slightly different results. This paranoiac shows an exaggerated response to both 5 mM Mg^{2+} (49 ± 7-sec continuous ciliary reversal (CCR), $n = 13$) and 10 mM Na^+ (62 ± 10-sec CCR, $n = 14$). Although also found to act as a partially dominant mutation in crosses to the wild type (E. AMBERGER and C. KUNG, unpublished results), F_1 cells from a *PaF* × *xntA*^{1} cross exhibited wild-type Mg^{2+} behavior (10 ± 3-sec CCR, $n = 32$) and Na^+ behavior (4 ± 1-sec CCR, $n = 32$). This cross was repeated two times, with similar results. Inducing autogamy in the F_1 yielded F_2 cells that segregated in a 1:1:1:1 ratio of wild type:paranoiac:eccentric:double mutant. The double mutant was weakly eccentric, often swimming backward briefly in Mg^{2+}, or turning repeatedly for 15–20 sec. Also, the double mutant exhibited abnormally prolonged backward swimming responses to K^+, Na^+ and Ba^{2+} (Table 5).

Eccentric was next crossed to k-shy A and k-shy B, two mutants whose behavioral phenotype suggests that they may have difficulties regulating intracellular Ca^{2+} concentration (EVANS *et al.* 1987). The F_1 from both crosses showed a wild-type response to Mg^{2+}, while F_2 cells segregated in a 1:1:1:1 ratio of wild type:k-shy:eccentric:double mutant (Table 5). The *ksA;xntA*^{1} and *ksB;xntA*^{1} double mutants behaved similarly in all our ionic test solutions, typically responding with whirling instead of backward swimming (Table 6).

fast-1A (*fA*) and fast-1B (*fB*) are two mutants that were isolated by virtue of their tendency to swim faster than the wild type in culture fluid (KUNG 1971). fast-1A was also reported to be slightly smaller than the wild type. Neither mutant exhibits obvious behavioral abnormalities in Ba^{2+}, Mg^{2+}, Na^+ or K^+ (Table 6). Both mutants were crossed to *xntA*^{1}. The resultant F_1 cells all swam in culture fluid at speeds approximating those of the wild type. The F_2s segregated in a 1:1:1:1 ratio of wild type:fast-1:eccentric:double mutant (Table 5). The *fA;xntA*^{1} double mutant was distinguished mainly by its small size, its high "resting" swimming speeds in culture, and its inability to respond behaviorally to Mg^{2+}. Similarly, *fB;xntA*^{1} double mutants were Mg^{2+}-insensitive and swam at characteristically high speeds in culture fluid.

Finally, eccentric was crossed to d4-521, a "K^+-resistant" mutant that grows in K^+ concentrations that are lethal to the wild type (SHUSTERMAN *et al.* 1978), and to d4-596, a "Ba^{2+}-shy" mutant that responds to millimolar Ba^{2+} by swimming backward for prolonged periods (SHUSTERMAN 1981). Although these mutants were suggested to be allelic to fast-1A described above (SHUSTERMAN 1981; S.-Y. CHANG and C. KUNG, unpublished), they both express archetypic eccentric swimming behavior in culture fluid, and both fail to swim backward in 5 mM Mg^{2+}. The results of these crosses are given in Table 2. F_1 cells from both crosses were Mg^{2+}-insensitive and Ni^{2+}-resistant, as were the F_2. The lack of wild-type cells among the F_2, despite a 1:1 segregation of the *nd6* marker, suggests that the three strains carry mutations in the same gene.

DISCUSSION

Eccentrics represent a class of Paramecium behavioral mutants that show a diminished response to Mg^{2+}. The phenotype results from recessive mutations at two unlinked loci, *xntA* (three alleles) and *xntB* (one allele). *xntA* mutants typically show little or no response to Mg^{2+} concentrations that cause the wild type to swim backward for 10 sec. They also exhibit a greatly reduced Mg^{2+} current in response to step changes in membrane potential under voltage clamp, consistent with their behavioral insensitivity to Mg^{2+}. Although the phenotype of *xntB* is weaker in terms of both Mg^{2+} behavior and Mg^{2+}-current amplitude (and also Ni^{2+} resistance), it is still clearly eccentric, suggesting that *xntA* and *xntB* affect the same intracellular component. The fact that the *xntA*^{1}*;xntB* double mutant is indistinguishable from the *xntA*^{1} single mutant supports this idea.

Eccentric behavior: Eccentric A exhibits several distinct behavioral anomalies in addition to its insensitivity to Mg^{2+}. Although characteristically abrupt, its avoidance reaction incorporates a backward swimming episode that is slightly longer than in the wild type. Also, it swims backward in 30 mM K^+ for almost twice as long as does the wild type. Perhaps most telling, whereas the wild type "dances" in Ba^{2+}, eccentric A swims backward for many 10s of seconds. These behaviors collectively suggest that the mutant has difficulties shutting off its depolarization-activated Ca^{2+} current and, indeed, this has been confirmed under voltage clamp (PRESTON and KUNG 1994). Not only does the Ca^{2+} current inactivate abnormally slowly during a step depolarization, it also takes longer to recover from this inactivation than does the wild-type current. This allows excessive amounts of Ca^{2+} to enter the cell and thereby causes the mutant to swim backward for prolonged periods. This behavior is highly reminiscent of dancer, a mutant that also suffers

from a Ca^{2+}-current inactivation defect (HINRICHSEN and SAIMI 1984). Genetic analyses have shown dancer and eccentric to be unrelated, however (Table 5). Eccentric A demonstrates a second significant ion-current anomally: the Ca^{2+}-dependent Na^+ current turns off (deactivates) much faster than normal. This may explain why recovery from Na^+-induced backward swimming is accelerated in the mutant compared with the wild type, and why the *xntA* mutation tends to reduce prolonged Na^+-induced backward swimming events associated with the pantophobiac and paranoiac mutations (Table 6).

Ni^{2+} resistance: The resistance of eccentric A to Ni^{2+} poisoning (Figure 2) is consistent with the loss of a major pathway for Ni^{2+} influx in this mutant (*i.e.*, I_{Mg}). I_{Mg} is weakened in eccentric B and, as expected, this mutant is weakly Ni^{2+}- resistant (Table 4). The resistance of pawn B to Ni^{2+} might be explained by the fact that depolarization triggers only weak Ca^{2+} influx in this mutant, and thus fails to activate the Ca^{2+}-dependent I_{Mg} fully (PRESTON 1990). Paranoiac F is also Ni^{2+}-resistant (Table 4) yet also expresses a robust Mg^{2+} current (R. R. PRESTON, unpublished): the cause of this discrepancy is uncertain at present.

The susceptibility of Paramecium to Ni^{2+} has been recognized for many decades (VON GELEI 1935), and has been analyzed extensively by ANDRIVON (1978). As reported here (Table 5), ANDRIVON found pawn B to be mildly Ni^{2+}-resistant, but he also reported fast 1B to remain motile in Ni^{2+} concentrations that paralyze the wild type. We found fast-1B to be similar to the wild type in terms of Ni^{2+} resistance (not shown): it is possible that fast-1B and fast-1A were confused in certain previous studies (see below).

Relationship between eccentric and other mutant strains: It seems likely that eccentric A has been isolated on at least two previous occasions. d4-526 was originally selected on the basis of its excessive backward swimming response to Ba^{2+}, resulting in its being given the moniker "Ba^{2+}-shy" (SHUSTERMAN 1981). A cross between d4-526 and eccentric A failed to yield wild-type cells among the F_2 progeny (Table 2) suggesting that the two cell lines carry mutations in the same gene. The behavioral and electrophysiological phenotype of d4-526 is indistinguishable from that of eccentric A (R. R. PRESTON, unpublished), so the observed abnormal response to Ba^{2+} again likely results from a reduced ability of the Ca^{2+} current to shut off during membrane depolarization (PRESTON and KUNG 1994).

Eccentric A is also allelic with d4-521, a K^+-resistant strain (SHUSTERMAN *et al.* 1978). Preliminary studies have confirmed that eccentric thrives in K^+ concentrations that kill the wild type, and also that K^+-resistant has a greatly reduced I_{Mg}. How K^+ resistance might be conferred on a cell is not understood, but might be related to reduced hyperpolarization-activated K^+-current amplitudes in these mutants (PRESTON and KUNG 1994). SHUSTERMAN *et al.* (1978) found that, in addition to being K^+-resistant, d4-521 is also adaptation-defective. As described above, transferring wild-type cells to a solution containing Ba^{2+} causes them to "dance." Prior growth in media containing high (>8 mM) concentrations of K^+ suppresses this response, however, a phenomenon which is known as "behavioral adaptation" (DRYL 1959). d4-521 (and eccentric A) is apparently unaffected by growth in high K^+, so it continues to dance when challenged with Ba^{2+}. Although the molecular mechanisms underlying adaptation are not understood, it is tempting to speculate that transferring cells to high K^+ causes sustained depolarization and thus prolonged activation of I_{Mg}. If intracellular free Mg^{2+} concentration rises significantly as a consequence, it is possible that this provides sufficient stimulus for the biochemical and electrophysiological modifications that constitute adaptation. This possibility is currently under investigation.

d4-521 was analyzed genetically by SHUSTERMAN (1981), who reported this strain to be allelic to fast-1A (*fA/fA*), the "fast-swimming" mutant isolated by KUNG (1971). Since d4-521 is also allelic to eccentric, a cross between eccentric and fast-1A would be expected to show that the two mutants are defective in the same gene. This expectation was not realized experimentally, however (Table 5): the two mutants were found to complement each other. The cause of this inconsistency between Shusterman's and the present data is uncertain, but probably results from a stock-keeping error in the years since SHUSTERMAN's original analysis. Interestingly, SHUSTERMAN (1981) also reported unpublished findings that fast-1A is allelic to Nir16p, a Ni^{2+}-resistant mutant isolated by ANDRIVON (1978). Nir16p was also reported to be K^+-resistant and Ba^{2+}-shy (SHUSTERMAN 1981), which would be consistent with Nir16p, d4-421 and eccentric being alleles of the same gene.

Eccentric gene products: The eccentric mutants were isolated by virtue of their inability to respond behaviorally to Mg^{2+}, a selection procedure designed to yield strains lacking an inward Mg^{2+} current. While the screen was largely successful (Figure 1), it seems unlikely that the mutations affect the Mg^{2+} permeability mechanism directly. The reasons for this assertion are discussed more fully in a companion report (PRESTON and KUNG 1994): in short, it is difficult to envision how the varied pleiotropic effects of the *xntA* mutations could result from loss of a single ion current. A more likely explanation is that the $xntA^+$ product is an intracellular regulator, such as a kinase, a phosphatase, or a G-protein, with the potential to affect simultaneously the functioning of several different ion channels. The data presented here offer no clues about the molecular identity of this regulator, but it is interesting to note that $xntA^1$, when combined with cam^{11}, causes an inordinate backward swimming response to Ba^{2+} (Table 5). cam^{11} causes a single amino acid substitution in the N-terminal lobe of

calmodulin (KINK *et al.* 1990) and, by itself, transforms the Ba^{2+} response from repeated avoidance reactions to 24 sec of backward swimming. *xntA*^{1} also causes prolonged backing (55 sec), but the *cam*^{11};*xntA*^{1} double mutant swims backwards for several minutes, far longer than can be explained by a simple additive effect of the two mutations. The exaggerated response appears specific for Ba^{2+}: the fact that the backward swimming episodes in Na^+ and K^+ are not prolonged similarly argues against this being a general effect on the ciliary reversal response. Moreover, the Ba^{2+} response appears specific for this particular calmodulin defect, since *cam*^{1} and *cam*^{3}, mutations that cause amino acid substitutions in the C-terminal lobe (KINK *et al.* 1990), fail to give the exaggerated Ba^{2+} responses when combined with *xntA*^{1} (Table 6). Taken together, these observations suggest that the *xntA*$^+$ product interacts with calmodulin, possibly through its N-terminal lobe, to control duration of ciliary reversal. The Ba^{2+} effect might be explained by a divalent cation binding step that is stimulated to a greater extent by Ba^{2+} than it is by other cations (such as Ca^{2+}, for example). Although this scenario remains highly speculative, it forms a framework for future investigations into the identity and functions of the *xntA* gene product. The resistance of eccentric A to both Ni^{2+} and K^+ is fortuitous, since it should allow us to isolate suppressor mutations that will help define the *xntA*$^+$ gene product's interactions *in vivo*.

This work was supported by grants from the National Institutes of Health (GM22714 and GM32386) and from the Lucille P. Markey Charitable Trust.

LITERATURE CITED

ALVAREZ-LEEFMANS, F. J., F. GIRALDEZ and S. M. GAMIÑO, 1987 Intracellular free magnesium in excitable cells: its measurement and biological significance. Can. J. Physiol. Pharmacol. **65:** 915–925

ANDRIVON, C., 1978 Etude du systeme de regulation de la perméabilité a l'ion nickel chez *Paramecium* et de ses rapports avec le chenal calcique d'excitation. Ph.D. Dissertation, University of Clermont II.

BEYENBACH, K. W., 1990 Transport of magnesium across biological membranes. Magnesium Trace Elem. **9:** 233–254

CHANG S.-Y., and C. KUNG, 1976 Selection and analysis of a mutant *Paramecium tetraurelia* lacking a behavioral response to tetraethylammonium. Genet. Res. **27:** 97–107.

DALEMANS, W., P. BARBRY, G. CHAMPIGNY, S. JALLAT, K. DOTT *et al.*, 1991 Altered chloride ion channel kinetics associated with the deltaF508 cystic fibrosis mutation. Nature **354:** 526–528.

DRUMM, M. L., D. J. WILKINSON, L. S. SMIT, R. T. WORRELL, T. V. STRONG *et al.*, 1991 Chloride conductance expressed by deltaF508 and other mutant CFTRs in *Xenopus* oocytes. Science **254:** 1797–1799.

DRYL, S., 1959 Effects of adaptation to environment on chemotaxis of *Paramecium caudatum*. Acta Biol. Exp. **19:** 83–93

EVANS, T. C., and D. L. NELSON, 1989 New mutants of *Paramecium tetraurelia* defective in a calcium control mechanism: genetic and behavioral characterizations. Genetics **121:** 491–500.

EVANS, T. C., T. M. HENNESSEY and D. L. NELSON, 1987 Electrophysiological evidence suggests a defective Ca^{2+} control mechanism in a new *Paramecium* mutant. J. Membr. Biol. **98:** 275–283.

FLATMAN, P. W., 1984 Magnesium transport across cell membranes. J. Membr. Biol. **60:** 1–14

FLATMAN, P. W., 1991 Mechanisms of magnesium transport. Annu. Rev. Physiol. **53:** 259–271

GRUBBS, R. D., 1990 Hormonal regulation of magnesium homeostasis in cultured mammalian cells. Metal Ions Biol. Syst. **26:** 177–192

GRUBBS, R. D., and M. E. MAGUIRE, 1987 Magnesium as a regulatory cation: criteria and evaluation. Magnesium **6:** 113–127

HENNESSEY, T. M., and C. KUNG, 1987 A calcium-dependent potassium current is increased by a single-gene mutation in *Paramecium*. J. Membr. Biol. **98:** 145–155

HINRICHSEN, R. D., and C. KUNG, 1984 Genetic analysis of axonemal mutants in *Paramecium tetraurelia* defective in their response to calcium. Genet. Res. **43:** 11–20.

HINRICHSEN, R. D., and Y. SAIMI, 1984 A mutation that alters properties of the calcium channel in *Paramecium*. J. Physiol. **351:** 397–410.

HINRICHSEN, R. D., Y. SAIMI and C. KUNG, 1984 Mutants with altered Ca^{2+}-channel properties in *Paramecium tetraurelia*: isolation, characterization and genetic analysis. Genetics **108:** 545–558.

HINRICHSEN, R. D., E. AMBERGER, Y. SAIMI, A. BURGESS-CASSLER and C. KUNG, 1985 Genetic analysis of mutants with a reduced Ca^{2+}-dependent K^+ current in *Paramecium tetraurelia*. Genetics **111:** 433–445.

KINK, J. A., M. E. MALEY, R. R. PRESTON, K.-Y. LING, M. A. WALLEN-FRIEDMAN *et al.*, 1990 Mutations in Paramecium calmodulin indicate functional differences between the C-terminal and N-terminal lobes *in vivo*. Cell **62:** 165–174.

KUNG, C., 1971 Genic mutants with altered system of excitation in *Paramecium aurelia*. II. Mutagenesis, screening and genetic analysis of the mutants. Genetics **69:** 29–45.

LEFORT-TRAN, M., K. AUFDERHEIDE, M. POUPHILE, M. ROSSIGNOL and J. BEISSON, 1981 Control of exocytotic processes: cytological and physiological studies of trichocyst mutants in *Paramecium tetraurelia*. J. Cell Biol. **88:** 301–311.

MAGUIRE, M. E., 1984 Hormone-sensitive magnesium transport and magnesium regulation of adenylate cyclase. Trends Pharmacol. Sci. **5:** 73–77

OERTEL, D., S. J. SCHEIN and C. KUNG, 1977 Separation of membrane currents using a Paramecium mutant. Nature **268:** 120–124.

PAPAZIAN, D. M., T. L. SCHWARZ, B. L. TEMPEL, Y. N. JAN and L. Y. JAN, 1987 Cloning of genomic and complementary DNA from *shaker*, a putative potassium channel gene from *Drosophila*. Science **237:** 749–753

PRESTON, R. R., 1990 A magnesium current in *Paramecium*. Science **250:** 285–288.

PRESTON, R. R., and C. KUNG, 1994 Inhibition of Mg^{2+} current by single-gene mutation in *Paramecium*. J. Membr. Biol. **139:** in press.

PRESTON, R. R., Y. SAIMI, E. AMBERGER and C. KUNG, 1990a Interaction between mutants with defects in two Ca^{2+}-dependent K^+ currents of *Paramecium tetraurelia*. J. Membr. Biol. **115:** 61–69.

PRESTON, R. R., M. A. WALLEN-FRIEDMAN, Y. SAIMI and C. KUNG, 1990b Calmodulin defects cause the loss of Ca^{2+}-dependent K^+ currents in two pantophobiac mutants of *Paramecium tetraurelia*. J. Membr. Biol. **115:** 51–60.

PRESTON, R. R., J. KINK, R. D. HINRICHSEN, Y. SAIMI and C. KUNG, 1991 Calmodulin mutants and Ca^{2+}-dependent channels in *Paramecium*. Annu. Rev. Physiol. **53:** 309–319.

PRESTON, R. R., Y. SAIMI and C. KUNG, 1992 Calcium current activated upon hyperpolarization of *Paramecium tetraurelia*. J. Gen. Physiol. **100:** 233–251.

RICHARD, E. A., R. D. HINRICHSEN and C. KUNG, 1985 A single gene mutation that affects a potassium conductance and resting membrane potential in *Paramecium*. J. Neurogenet. **2:** 239–252.

ROJAS, C. V., J. WANG, L. S. SCHWARTZ, E. P. HOFFMAN, B. R. POWELL *et al.*, 1991 A Met-to-Val mutation in the skeletal muscle Na^+ channel α-subunit in hyperkalaemic periodic paralysis. Nature **354:** 387–389.

ROMANI, A., and A. SCARPA, 1992 Regulation of cell magnesium. Arch. Biochem. Biophys. **298:** 1–12

SAIMI, Y., 1986 Calcium-dependent sodium currents in *Paramecium*: mutational manipulations and effects of hyper- and depolarization. J. Membr. Biol. **92:** 227–236.

SAIMI, Y., and C. KUNG, 1987 Behavioral genetics of *Paramecium*. Annu. Rev. Genet. **21:** 47–65.

SAIMI, Y. and K.-Y. LING, 1990 Calmodulin activation of calcium-dependent sodium channels in excised membrane patches of *Paramecium*. Science **249:** 1441–1444.

SAIMI, Y., R. D. HINRICHSEN, M. FORTE and C. KUNG, 1983 Mutant analysis shows that the Ca^{2+}-induced K^+ current shuts off one type of excitation in *Paramecium*. Proc. Natl. Acad. Sci. USA **80:** 5112–5116

SATOW, Y., and C. KUNG, 1980 Membrane currents of pawn mutants of the *pwA* group in *Paramecium tetraurelia*. J. Exp. Biol. **84:** 57–71.

SATOW, Y., S.-Y. CHANG and C. KUNG, 1974 Membrane excitability: made temperature-dependent by mutations. Proc. Natl. Acad. Sci. USA **71:** 2703–2706

SHUSTERMAN, C. L., 1981 Potassium-resistant mutants and adaptation in *Paramecium tetraurelia*. Ph.D. Dissertation, University of Wisconsin-Madison.

SHUSTERMAN, C. L., E. W. THIEDE and C. KUNG, 1978 K^+-resistant mutants and "adaptation" in *Paramecium*. Proc. Natl. Acad. Sci. USA **75:** 5645–5649.

SONNEBORN, T. M., 1970 Methods in *Paramecium* research. Methods Cell Physiol. **4:** 241–339.

VAN HOUTEN, J., S.-Y. CHANG and C. KUNG, 1977 Genetic analysis of "paranoiac" mutants of *Paramecium tetraurelia*. Genetics **86:** 113–120.

VON GELEI, J., 1935 Ni Infusorien im Dienste der Forschung und der Unterrichtes. Biol. Centrabl. **505:** 57–74

WHITE, R. E., and H. C. HARTZELL, 1989 Magnesium ions in cardiac function. Regulator of ion channels and second messengers. Biochem. Pharmacol. **38:** 859–867

Communicating editor: S. L. ALLEN