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Genetic analysis of axonemal mutants in *Paramecium tetraurelia* defective in their response to calcium

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SUMMARY

Six axonemal mutants of *Paramecium tetraurelia* have been isolated that are unable to respond properly to calcium. The mutants, designated atalantas, cannot swim backward when stimulated by ions or heat. Genetic analyses reveal that all six mutants are recessive and fall into four complementation groups. Three of the mutants in one complementation group are phenotypically non-leaky, one is leaky and two are extremely leaky, only displaying their phenotypes at elevated temperatures. The complete mutants, *ataA*, are also abnormal in their forward swimming. This abnormality co-segregates with the inability to swim backward. *ataA*^{1} is not allelic to several membrane mutants of *P. tetraurelia*.

1. INTRODUCTION

The activity of eukaryotic cilia and flagella is controlled by calcium ions. Changes in the internal concentration of Ca^{2+} may have various consequences, e.g. (1) a reversal in the direction of flagellar wave propagation in the trypanosome *Crithidia* (Holwill & McGregor, 1975), (2) changes in the flagellar waveform during photo-stimulation of *Chlamydomonas* (Schmidt & Eckert, 1976), (3) the arrest of gill ciliary beat in the mussel *Elliptio* (Satir, 1975) and (4) the asymmetry of the bending waves in sperm flagella that leads to quiescence (Gibbons & Gibbons, 1980). In *Paramecium*, the depolarization of the cell membrane by various stimuli opens Ca^{2+} channels on the ciliary membrane and Ca^{2+} ions flow down their electrochemical gradient into the cilium. The internal Ca^{2+} causes an increase in the frequency of ciliary beating and a reversal in the direction of the ciliary power stroke that results in backward swimming (Naitoh & Eckert, 1974; Kung & Saimi, 1982).

The site and mechanism of action of Ca^{2+} within the cilium is unknown. The fact that detergent-treated cells (Naitoh & Kaneko, 1972) and isolated axonemes (Bessen *et al.* 1980) the 9+2 microtubular ensemble and its associated structures excluding the membrane) still respond to Ca^{2+} indicates that the cell membrane is not the site of action. Paralysed mutants of *Chlamydomonas* have been employed to identify components of the axoneme required for flagellar beat formation and propagation (Huang *et al.* 1981; Witman *et al.* 1978), but these mutants cannot be used to study the problem of Ca^{2+} action on the axoneme directly.

The present work describes the isolation and genetic analysis of six mutants of

Paramecium tetraurelia, designated atalantas, that display an altered axonemal response to Ca^{2+}; they are unable to reverse the direction of their ciliary beat. A preliminary study on the first such mutant, atalanta A^1 (d4-148), has been reported previously (Kung *et al.* 1975). Electrophysiological studies have shown that the membranes of the mutants are normal. Partial demembranation, which allows free access of Ca^{2+} ions to the axoneme, does not restore the ability of the mutants to reverse their ciliary beat (Hinrichsen *et al.* 1983). These mutants offer the possibility of uncovering regulatory elements on the axoneme that are responsible for the Ca^{2+}-induced changes in ciliary beat form, frequency and direction.

2. MATERIALS AND METHODS

(i) *Stocks and culture conditions*

We used *Paramecium tetraurelia*, stock 51s (kappa-free), d4-90 paranoiac A (*PaA/PaA*), d4-91 fast-2 (*fna/fna*), d4-93 deformed body (*bd/bd*), d4-94 pawnA (*pwaA/pwA*), d4-95 pawn B (*pwB/pwB*), d4-131 pawn C (*pwC/pwC*) (Kung, 1979), the first axonemal mutant d4-148 atalanta A^1 (*ataA*^{1}/*ataA*^{1}) (Kung *et al.* 1975) and the more recently isolated axonemal mutants d4-613 atalanta A^2 (*ataA*^{2}/*ataA*^{2}), d4-614 atalanta A^3 (*ataA*^{3}/*ataA*^{3}), d4-612 atalanta B (*ataB/ataB*), d4-615 atalanta C (*ataC/ataC*), and d4-616 atalanta D (*ataD/ataD*). The *ataA* mutants are non-leaky, *ataB* is leaky and *ataC* and *ataD* are extremely leaky. Paramecia were cultured at 28 °C in Cerophyl medium enriched with stigmasterol (5 mg/l) buffered with sodium phosphates and bacterized with *Enterobacter aerogenes* (Sonneborn, 1970).

(ii) *Crosses*

Standard techniques of obtaining F_1 through conjugation and F_2 through autogamy in *P. tetraurelia* were employed as described by Sonneborn (1970). The axonemal mutants were crossed to d4-93, a recessive mutant with a deformed body, and the two exconjugants were separated into culture wells. After the cells had undergone 8–10 fissions the F_1 phenotypes were scored. Further isolations were made until the clones had grown for more than 20 fissions, after which autogamy can be induced. When 95–100 % of the cells of each clone had undergone autogamy, F_2 cells were isolated, grown up and scored for their behaviour and body shape. Conjugation of different types leads to heterozygosity, but autogamy makes the genome completely homozygous at all loci (Sonneborn, 1970). Thus, for a one-factor cross of a/a × +/+, the F_1 resulting from conjugation is +/a and the autogamous F_2s segregate as a/a : +/+, 1:1. For a cross of two unlinked factors a/a +/+ × +/+ b/b, the F_1 is +/a +/b and the autogamous F_2s segregate as +/+, +/+ : a/a, +/+ : +/+, b/b : a/a, b/b 1:1:1:1. Linkage can distort this ratio.

Complementation tests were done by crossing an atalanta mutant carrying the deformed-body marker to another atalanta mutant with a normal body. The F_1 phenotype was scored when neither exconjugant showed the recessive deformation trait. The cells were allowed to undergo autogamy and the F_2 behaviour and body morphology phenotypes were determined to ensure that cross-fertilization had indeed taken place.

(iii) *Mutagenesis*

Mutagenesis was carried out on cultures that had undergone more than 20 fissions since the last autogamy using N-methyl-N′-nitro-N-nitrosoguanidine (Kung, 1971). After treatment, the cells were immediately separated into eight groups and induced to undergo autogamy. This separation into eight groups allows us to distinguish more clearly mutants of different origins. The cells were tested for exautogamous death and used only when 50–65% of the cells died after autogamy. Cells were then allowed to grow for 6–8 fissions after autogamy to overcome phenomic lag before the selection of mutants was initiated.

(iv) *Selection of mutants*

Two different methods were employed to isolate axonemal mutants. The first method was used to isolate axonemal mutants that expressed their phenotype at room temperature. The method was the same as that described by Kung (1971) to isolate mutants unable to perform avoiding reactions. Cells were placed at the bottom of a vertical glass column filled with a solution that induces avoiding reactions; cells with an impaired ability to swim backward reached the top of the column sooner than wild-type cells. Both axonemal mutants and those that are unable to induce action potentials (pawns and others) were enriched using this technique.

The second method was used to enrich for axonemal mutants that expressed their phenotype at elevated temperatures. A glass column, 60 cm high and 2 cm in diameter, was filled with a solution comprised of 4 mM KCl, 8 mM NaCl, 1 mM $CaCl_2$, 10^{-2} mM EDTA and 1 mM HEPES, pH 7·2. Plastic tubing with 50 °C water circulating through it was wrapped around the column in such a manner as to heat the column in a graduated fashion, with a temperature range of 22 °C at the bottom and 40 °C at the top. Wild-type *Paramecium* has a negative geotaxis and a thermotaxis (Hennessey & Nelson, 1979) and tends to avoid strongly the top of the column; axonemal mutants, including those that are temperature sensitive, would not be able to avoid the heated upper portion of the column. Only those cells that reached the top of the column were collected, cloned and re-examined.

(v) *Behavioural assays*

Methods used to describe the behaviour of cells were the same as those described by Hinrichsen *et al.* (1983). Cells were placed in a control solution (1 mM K^+, 1 mM Ca^{2+}, 1 mM HEPES, 10^{-2} mM EDTA, pH 7·2) for 5 minutes before being transferred to a solution of different ionic composition, and their behaviour was observed for up to 3 minutes. The temperature-sensitive phenotypes were observed on a heated stage (Cambion temperature regulator).

3. RESULTS

(i) *Phenotypes*

The six atalanta mutants have an impaired ability to swim backward when stimulated by ions or heat. Three of the mutants, *ataA*^{1}, *ataA*^{2} and *ataA*^{3}, cannot swim backward at all. In conditions where the wild-type cells swim backward for seconds or minutes, these mutants spin in place or move forward slowly while spinning rapidly. The three mutants can be distinguished from one another by their forward swimming behaviour. *ataA*^{1} swims at approximately the same rate as wild type; *ataA*^{2} swims slowly, during stationary phase many cells appear immobile; *ataA*^{3} swims at approximately the same rate as wild type during log phase but becomes extremely sluggish during the stationary phase of growth.

Table 1. *F_1 phenotypes and autogamous F_2 segregations of crosses between atalanta mutants and the deformed-body mutant*

		Autogamous F_2				
Cross†	F_1 phenotype	Wild	*ata*	*bd*	*ata* and *bd*	*P*‡
ataA^1 × *bd*	Wild type	26	27	32	21	0·50
ataA^2 × *bd*	Wild type	30	15	23	26	0·08
ataA^3 × *bd*	Wild type	51	39	49	35	0·20
ataB × *bd*	Wild type	54	47	55	34	0·15
ataC × *bd*	Wild type	41	49	43	47	0·70
ataD × *bd*	Wild type	40	29	31	32	0·60

† Symbols used in the crosses: *bd* has a deformed body but is normal in its response to simulation. *ata* is the axonemal mutation that has a normal body shape. Wild type has both a normal body shape and response to Ca^{2+}. *Paramecium* is diploid. For simplicity, the allelic symbols are used to stand for the genotypes of the lines, e.g. *ataA*^{1} × *bd* stands for *ataA*^{1}/*ataA*^{1}, +/+ × +/+, *bd*/*bd*.

‡ In a cross, without marker linkage, the F_2 obtained by autogamy should segregate in a 1:1:1:1 ratio of wild type: *ata*:*bd*:*ata* and *bd*. χ^2 values were calculated using such an expected ratio.

The three remaining mutants are leaky. When stimulated, *ataB* swims backward very briefly, then spins in place. *ataC* and *ataD* are nearly normal at room temperature, swimming backward for only slightly shorter duration than the wild type; *ataC* and *ataD* cannot be distinguished phenotypically. These leaky mutants best express their phenotypes when the temperature is raised. At 39 °C, all mutants immediately are unable to swim backward. Details concerning the behavioural phenotypes, electrophysiological examinations, and demembranated model experiments are described elsewhere (Hinrichsen *et al.* 1983).

(ii) *Genetic analyses*

Each mutant was crossed to the marker line, d4-93, a recessive deformed-body mutant that behaves normally. All six heterozygous F_1s were able to swim backward, indicating that each mutant carries a recessive mutation (Table 1). The

F_1s were induced to undergo autogamy and the F_2s were scored for their phenotypes. F_2s from all six crosses segregate in an approximate 1:1:1:1 ratio for the behavioural and deformed-body traits (Table 1). These data indicate that all six mutants carry single-site, genic lesions that are unlinked to the deformed-body locus. Furthermore, the altered forward swimming speeds of *ataA*^{1}, *ataA*^{2} and *ataA*^{3} all co-segregated in the F_2 with the inability to swim backward. This indicates that a single locus, or two very closely linked loci, may control both ciliary beat frequency and direction.

Table 2. *F_1 phenotypes and autogamous F_2 segregations of crosses between atalanta mutants*†

	Cross‡	F_1 phenotype	F_2 phenotypes	*P*§
1	*ataA*^{1} × *ataA*^{2}	*ataA*^{2}	*ataA*^{1}:*ataA*^{2}:+ 44:43:0	0·99
2	*ataA*^{1} × *ataA*^{3}	*ataA*^{3}	*ataA*^{1}:*ataA*^{3}:+ 81:81:2	0·98
3	*ataA*^{2} × *ataA*^{3}	*ataA*^{2}	*ataA*^{2}:*ataA*^{3}:+ 52:44:0	0·65
4	*ataA*^{1} × *ataB*	Wild type	*ataA*^{1}:*ataB*:+ 80:29:42	0·30
5	*ataA*^{1} × *ataC*	Wild type	*ataA*^{1}:*ataC*:+ 74:38:40	0·92
6	*ataA*^{1} × *ataD*	Wild type	*ataA*^{1}:*ataD*:+ 78:30:36	0·60
7	*ataA*^{3} × *ataB*	Wild type	*ataA*^{3}:*ataB*:+ 44:28:28	0·50
8	*ataA*^{3} × *ataC*	Wild type	*ataA*^{3}:*ataC*:+ 84:59:52	0·10
9	*ataA*^{3} × *ataD*	Wild type	*ataA*^{3}:*ataD*:+ 38:27:24	0·30
10	*ataB* × *ataC*	Wild type	*ataB*:*ataC*:+ 66:42:40	0·40
11	*ataB* × *ataD*	Wild type	*ataB*:*ataD*:+:*ata** 52:36:41:56	0·06
12	*ataC* × *ataD*	Wild type	*ata*:+ 80:32	0·75

† All crosses carried the deformed-body marker. In all crosses, this marker segregated in a 1:1 ratio in the F_2 (data not shown).

‡ For simplicity, the allelic symbols are used to stand for the diploid genotypes of the lines. In the one-factor cross (1), for example, *ataA*^{1} × *ataA*^{2} stands for *ataA*^{1}/*ataA*^{1} × *ataA*^{2}/*ataA*^{2}; in the two-factor cross (4), for example, *ataA*^{1} × *ataB* stands for *ataA*^{1}/*ataA*^{1}, +/+ × +/+, *ataB*/*ataB*.

§ χ^2 values are calculated with an expected ratio of 1:1:0 for crosses 1–3, 2:1:1 for crosses 4–10, 1:1:1:1 for cross 11, and 3:1 for cross 12.

Crosses between the atalanta mutants were performed to determine the number of loci represented by this collection of mutants. The results (Table 2) indicate that there are four complementation groups and four loci. The first group contains the three phenotypically non-leaky mutants *ataA*^{1}, *ataA*^{2} and *ataA*^{3}, the second group consists of the leaky mutant *ataB*, while the third and fourth groups are the two extremely leaky mutants *ataC* and *ataD*, respectively. The three members of the

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first group, *ataA*, can be distinguished phenotypically by their forward swimming speed as indicated above. When they are crossed to one another, the F_1 cells remain unable to swim backward. The dominance relationship of the three allelic variants in terms of forward swimming speed is $ataA^2 > ataA^3 > ataA^1$. The F_2s resulting from the three crosses show a 1:1 segregation of each allele (Table 2), except in the case of $ataA^1 \times ataA^3$, where two wild-type clones appeared out of 165 F_2 clones isolated. These two lines may have arisen from intragenic recombination between the $ataA^1$ and $ataA^3$ alleles.

Table 3. *Determination of the genotype of* ata*

	Cross†	F_1 phenotype	F_2 phenotypes: wild type: *ataB*:*ataD*:*ata**	P‡
1	*ata** × wild type	wild type	41:52:36:56	0·15
2	*ata** × *ataB*	*ataB*	0:37: 0:43	0·50
3	*ata** × *ataD*	*ataD*	0: 0:29:34	0·55

† The results lead to the conclusion that *ata** is the double mutant. Thus the genotypes of the crosses are: (1) *ataB*/*ataB*, *ataD*/*ataD* × +/+, +/+, (2) *ataB*/*ataB*, *ataD*/*ataD* × *ataB*/*ataB*, +/+, and (3) *ataB*/*ataB*, *ataD*/*ataD* × +/+, *ataD*/*ataD*.

‡ χ^2 values are calculated with an expected ratio 1:1:1:1 for the first cross, 1:1 for the second and third crosses.

In all but one of the crosses between complementation groups the F_1 phenotype was normal and there was a 1:3 (wild type:mutant types) ratio in the F_2, a ratio expected of unlinked loci. However, in one cross an F_2 phenotype appeared that was different from both parents and wild type. When *ataB* (leaky) is crossed to *ataD* (extremely leaky) there is an approximate 1:1:1:1 segregation of wild type:leaky:extremely leaky:non-leaky. The phenotypically non-leaky F_2 cells (designated *ata** in Table 2) never exhibited any detectable backward swimming by any type of stimulation; they would spin in place or swim forward slowly while spinning. The segregation ratio suggests that *ata** was a double mutant of *ataB* and *ataD*. In order to test this hypothesis *ata** was crossed to wild type (Table 3). The F_1 of the backcross is wild type and the F_2 again gives an approximate 1:1:1:1 segregation ratio of wild type:*ataB*:*ataD*:*ata**, indicating that *ata** indeed carries both the *ataB* and *ataD* mutations. This conclusion was further confirmed by test-croses of *ata** to *ataB* or *ataD* (Table 3). In no other intergenic cross was there a detectably new phenotype when double mutants were constructed; the double mutant showed the phenotype of the more severe defect. Thus, the four loci are unlinked and their interaction follows an epistatic series of non-leaky > leaky > extremely leaky (*ataA* > *ataB* > *ataC* = *ataD*).

One of the atalanta mutants, $ataA^1$, was crossed to several behavioural mutants defective in membrane properties to determine whether they were allelic and the phenotypes of double mutants. Paranoiac A (genotype *PaA*/*PaA*) carries a dominant mutation that causes an overreaction to Na^+ and spontaneous long backward swimming in culture fluid (Kung *et al.* 1975). *PaA* was crossed to $ataA^1$ and the resulting F_1 was partially paranoiac (Table 4), while the F_2 segregated in a 1:1:1:1 ratio of *PaA*, $ataA^1$:*PaA*:$ataA^1$:wild type. The double mutant

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(*PaA/PaA ataA*^{1}/*ataA*^{1}) swims backward slightly when stimulated in 20 mM Na^+ before it stops and spins in place. Fast-2 (*fna/fna*) carries a recessive mutation that results in a normal response to all ions except that it does not react to Na^+ (Kung *et al.* 1975). When *fna* is crossed to *ataA*^{1}, the F_1 is wild type and there is a 1:1:1:1 segregation in the F_2 of *fna ataA*^{1}:*fna*:*ataA*^{1}:wild type (Table 4). The double mutant behaves like *ataA*^{1} to all stimuli except Na^+ where, like the *fna* parent, it does not show any reaction. *ataA*^{1} was crossed to the three pawn mutants, pawn

Table 4. *F_1 phenotypes and autogamous F_2 segregations of crosses between* ataA^1 *and other behavioural mutants of* P. tetraurelia*

	Cross†	F_1 phenotype	F_2 genotypes	*P*‡
1	*ataA*^{1} × *PaA*	Partial paranoiac	*ataA*^{1}, *PaA*:*ataA*^{1}:*PaA*:+ 43:55:46:35	0·20
2	*ataA*^{1} × *fna*	Wild type	*ataA*^{1}, *fna*:*ataA*^{1}:*fna*:+ 45:51:42:49	0·70
3	*ataA*^{1} × *pwA*	Wild type	*pw*:*ata*:+ 45:19:30	0·40
4	*ataA*^{1} × *pwB*	Wild type	*pw*:*ata*:+ 46:26:24	0·90
5	*ataA*^{1} × *pwC*	Wild type	*ataA*^{1}, *pwC*:*ataA*^{1}:*pwC*:+ 47:57:42:41	0·25

* All crosses carried the deformed-body marker. In all crosses, this marker segregated in a 1:1 ratio in the F_2 (data not shown).

† For simplicity, the allelic symbols are used to stand for the genotype of the lines, e.g. in cross 1, *ataA*^{1} × *PaA* stands for *ataA*^{1}/*ataA*^{1}, +/+ × +/+, *PaA/PaA*.

‡ χ^2 values are calculated with an expected ratio of 1:1:1:1 in crosses 1, 2 and 5, and 2:1:1 in crosses 3 and 4.

A (*pwA*), pawn B (*pwB*) and the temperature-sensitive pawn C (*pwC*). Pawn mutants are defective in their function of the Ca^{2+}-channel (Kung, 1979). In all three cases the F_1 generation was wild type and the F_2 showed a segregation ratio of 2:1:1 for *pw*:*ataA*^{1}:wild type. The double mutant always behaves like the pawn mutant. In the cases of the pawn–atalanta double mutants the pawn phenotype appears epistatic behaviourally because pawn mutants totally lack any response to stimuli. Unlike the atalantas they do not even whirl or spin in place. These results indicate that mutations affecting membrane functions and the one affecting axonemal functions (*ataA*) concern different genes, as expected because different gene products reside on the membrane and the axoneme. Since such results are expected and not informative, the study of the non-identity between genes governing membrane functions and genes for axonemal functions was not extended to *ataB*, *ataC* and *ataD*.

4. DISCUSSION

Axonemal mutants have been reported in *Chlamydomonas* (Huang *et al.* 1981; Witman *et al.* 1978) and in humans (Sturgess *et al.* 1979) in which the flagella or cilia are unable to beat. These mutants have gross structural defects in the flagella or cilia, i.e. major axonemal components are missing, resulting in paralysis. A

backward swimming mutant of *Chlamydomonas* has been described (Nakamura, 1979) that cannot swim forward, and may be axonemal in nature. There is no reported mutant, however, that is motile but is unable to respond properly to shifts in Ca^{2+} concentration. The atalanta mutants described in this paper may in fact be Ca^{2+}-response mutants (Hinrichsen *et al.* 1983). An analysis of the Ca^{2+} inward currents under voltage clamp has revealed that the mutants are normal in terms of the maximum inward current, time to peak and the V_{max}. The extremely leaky mutants *ataC* and *ataD* also were shown to have normal membrane properties when analysed at 38 °C, where their phenotype is completely expressed. Therefore the mutants have a normal Ca^{2+} influx into the cell upon depolarization. When the membrane of a wild-type cell is partially disrupted so as to allow Ca^{2+} free access to the axoneme the cells will swim backward at the proper [Ca^{2+}] (Naitoh & Kaneko, 1972). The atalanta mutants, however, only spin rapidly in place when their membranes are partially disrupted. These data indicate that the atalanta mutants cannot respond properly to Ca^{2+} once it enters the cell. The atalanta mutants are distinct from the pawn mutants that are unable to respond to stimuli (Kung *et al.* 1975). The pawn lesion resides in the membrane and will not allow Ca^{2+} influx upon depolarization; demembranation in the presence of Ca^{2+} causes the pawn cells to swim backward (Kung & Naitoh, 1973). Therefore the lesion in the atalanta mutants is not in the membrane, but rather in the inability of the axoneme to respond to Ca^{2+} in a normal manner. Electron microscopic examinations of the thin-sectioned mutants' axonemal structure reveals no gross defect in the axoneme (J. Peterson, R. Dute, C. Schobert, N. Pollack and C. Omoto, unpublished data).

The six mutants presented here represent four unlinked loci, with each mutation being recessive; *ataA* was the only non-leaky locus found, *ataB* is slightly leaky and *ataC* and *ataD* are extremely leaky mutants. The phenotypic differences and the degree of leakiness suggest that *ataA* to *ataD* affect different components in the machinery for the Ca^{2+}-response. The only double mutant to give a phenotype different from that of either single mutant parent involved *ataB* and *ataD*. While both parents are leaky, the double mutant is non-leaky. The Ca^{2+} influx, as determined by voltage clamp, is normal in the double mutant (Y. Saimi, unpublished data) and an electron microscopic examination found no detectable axonemal component missing (J. Peterson, unpublished data). Therefore the *ataB ataD* double mutant is not different from the phenotypically complete *ataA* in phenotype. All other double mutants between the four loci showed the phenotype of the more severe defect. The simplest explanation of this observation is that these *ata* gene products work in series such that the pathway is limited by the most severely restricted step. The *ataB* and *ataD* interaction would be an exception to this scheme.

The $ataA^1$ mutant showed no allelic relationship to the membrane mutants tested. In the case of the Paranoiac A (*PaA*), the *PaA* $ataA^1$ double mutant gave a prolonged response to low Na^+ stimulation (the *PaA* phenotype) but it merely spins in place (the $ataA^1$ phenotype) for the same length of time. When $ataA^1$ is crossed to fast-2 (*fna*) or the pawn mutants (*pwA*, *pwB* or *PwC*) the membrane mutant phenotype was expressed. These results are predicted because the membrane mutants control the amount and the time course of Ca^{2+} influx. A mutant like pawn

has no or very little Ca^{2+} inward current and is expected to be epistatic over the atalanta mutants.

It is interesting that the *ataA* mutants can be distinguished by their forward swimming speed. *ataA*[1] swims at a rate comparable to the wild type, *ataA*[2] is a very slow swimmer and *ataA*[3] is a growth-phase-dependent slow swimmer. All three mutants swim with an exaggerated spiral, but the degree of spiralling is greatest in *ataA*[2] and least in *ataA*[1]. The impaired forward-swimming behaviour of the *ataA* mutants co-segregates with the inability to swim backward, i.e. in none out of over 900 F_2 segregants with *ataA* phenotypes (Tables 1 and 2) were the two traits found to segregate. The possibility of two separate but closely linked loci, one for the forward-swimming phenotype and one for the backward-swimming phenotype, is especially remote since that would imply that *ataA*[1], *ataA*[2] and *ataA*[3] are all double mutants of the same two loci. The direct inference for a slower swim speed is a slower ciliary beat and a wider spiralling path from a change in beat direction. Our finding that forward swim speed and path are governed by a gene which also governs the ability to swim backward strongly suggests that the *ataA* gene product is used in both forward and backward swimming. Revertants of *ataA*[3] have been isolated (R. Hinrichsen, unpublished data) that swim backward slightly, but have a greater swimming speed than wild-type cells. Such mutants may be useful in identifying other genes that regulate ciliary beat frequency.

Slow-swimming mutants of *P. tetraurelia* have been reported (Kung, 1979). These mutants, designated sluggish, are extremely slow growing and have the ability to swim backward at a slow rate (R. Hinrichsen, unpublished observations). There is also a slow-swimming mutant of *P. caudatum* (Takahashi & Naitoh, 1978) that swims at approximately 70% the rate of wild type. Further examination has revealed a decrease in the amount of ATPase activity of 14s dynein (Hayashi & Takahashi, 1979). Whether the *ataA* mutants have an altered dynein ATPase activity is unknown. A biochemical analysis of the axonemal components in these mutants will better enable us to identify the lesions in the ciliary reversal mechanism.

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