MUTANTS OF PARAMECIUM DEFECTIVE IN CHEMOKINESIS TO FOLATE

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ABSTRACT

Ten mutant lines of Paramecium tetraurelia defective in attraction to folate were isolated and examined. All mutants were normal in response to other attractants and repellents tested. One mutant was able to accumulate in folate given sufficient time. All mutations were recessive and behaved as single site Mendelian lesions. Complementation tests indicate that the mutants fall into three complementation groups. Mutants of Group 2 fall into two phenotypic classes and probably represent two alleles of the mutated fo12 gene. Possible sites of the mutants' blocks in chemoresponse are discussed.

Paramecia detect and respond to some soluble chemicals in their environment. These unicells swim by means of beating cilia. Changes in swimming behavior are part of their characteristic responses to the detection of chemicals. Changes in the individual cells' swimming behavior, in turn, lead to alterations in population behavior, that is, in accumulation or dispersal. Hence, chemosensory transduction can be studied on various levels in paramecia (Figure 1): the level of the population, individual motor behavior, or even membrane electrical changes because ciliary beating determines swimming behavior and ciliary beating is under membrane electrical control (Eckert 1972; Machemer 1976). The study of these various levels can be facilitated by the generation of mutants to perturb one pathway component at a time. Comparison of mutant with normal individual cells and populations can lead to identification of the altered component.

In order to understand the primary steps of the chemosensory transduction pathway in Figure 1, we generated and selected mutants unable to detect or respond to folate, an attractant to wild-type paramecia. Ten mutant lines were isolated as described below and analyzed genetically and behaviorally. The ten lines fall into three complementation groups and none of these lines appears to be defective in response to any attractants or repellents other than folate. Therefore, these mutants are likely to have defects early in the chemosensory transduction response pathway, that is, in the part of the pathway specific for the detection of folate. The analyses of phenotypes and genotypes of these mutants are presented below. The possible sites of the mutant lesions discussed include the possibility of defects in a chemoreceptor for folate.

MATERIALS AND METHODS

Strains: All strains belong to Paramecium tetraurelia (previously called P. aurelia species 4; see Sonneborn 1970) stock 51-s (endosymbiont free) or are mutants derived from stock 51-s. Mutant

Figure 1.—Schematized chemosensory pathway in Paramecium. Chemical cues contact putative receptors, perhaps at the cell membrane; chemical cue is transduced into electrical cue that affects the motor organelles, cilia; altered ciliary movement of individuals indirectly causes accumulation and dispersal of a population.

Lines include the ten behavioral mutants unable to normally respond to folate and strain d4-93, which contains a behaviorally normal, body-deformed mutation (bdB) used as a genetic marker here.

Culturing: Cells are routinely grown in overnight cultures of Klebsiella pneumoniae in Cerophyl rye grass medium.

Solutions: All solutions are buffers containing 1 mM Ca(OH)$_2$, 1.3 mM Tris (hydroxymethyl) aminomethane, 1 mM citric acid and the indicated salts at pH 7.02, with the exception of 1 mM KOH solution at pH 8.6.

Assays of chemokinesis

T-maze assay: The T-maze is a modified three-way stopcock with a two-way plug (see Van Houten et al. 1975). Test solution fills one arm of the T; control solution fills the other arm. The plug bore is filled with cells in control solution. The assay starts when the stopcock bore is turned to connect the test and control arms. Cells are allowed to distribute themselves between the two arms for 30 min at which time the stopcock is turned to seal the arms, the arms are emptied and the number of cells in each arm counted. The index of attraction and repulsion is $I_{che}$ (number of cells in test arm/total cells in test and control arms). $I_{che} > 0.5$ indicates attraction; $I_{che} < 0.5$ indicates repulsion from the test solution.

Well test: Three small diameter wells (two 0.5-cm outer and 0.6-cm inner wells) in 0.5 cm Plexiglas are connected by short canals (Figure 2). Glass coverslips serve as bottoms of the wells. Control solution and test solution are pipetted into the two side wells and canals. At least 50 cells and control solution are pipetted into the middle well until the solutions make contact with the solutions in the canals. Cells immediately begin to distribute themselves into the side wells and the number in test and control wells are scored between 1 and 2 min from the start of the test. When at least twice as many cells are in test as control solution, the test is scored +; even distribution between wells is scored 0; at least twice as many cells in control as test well is scored -.

Mutagenesis: Cultures that had undergone about 20 fissions since the last autogamy were mutagenized by exposure to N-methyl-N'-nitro-N-nitrosoguanidine (Sonneborn 1970; Kung 1971). Treated cells were distributed to flasks to grow, starve and undergo autogamy. After autogamy, cells were again fed and grown for 5-10 fissions to allow for phenomic lag before the cells were screened for folate-insensitive mutants. [Sonneborn (1947) confirmed that P. tetraurelia is capable of undergoing autogamy, a process by which the micronucleus reorganizes and becomes completely homozygous.]

Screening for mutants: Two procedures were used. One mutant line (fol$^{d6}$) was identified using Procedure 1; nine mutant lines were identified using Procedure 2.

Procedure 1 is an enrichment process. Cells were tested in a T-maze with 1 mM K$_2$ folate and 2 mM KCl (control) solutions. All cells that migrated into the KCl control were grown in culture fluid and the procedure was repeated with these cells. After four repetitions to enrich for mutants, single cells were cloned and tested for the ability to be attracted to folate in the T-maze.

Procedure 2 utilized two two-way stopcocks connected to form three chambers (Figure 3). Control solution filled one chamber; an exponential gradient of 5 mM KCl control and 2.5 mM K$_2$ folate filled the middle chamber; 2.5 mM K$_2$ folate with cells filled the last chamber. The stopcocks between chambers were opened and the mutagenized cells were allowed to distribute themselves in the gradient. Cells swimming down the exponential gradient of attractant into the chamber with control solution were cloned and tested in the well test and eventually tested in the T-maze.
MUTANTS DEFECTIVE IN CHEMOKINESIS

FIGURE 2.—Well test apparatus. Test and control solutions filled the side wells; cells in control solution filled the middle well. Cells distributed between the three wells. At least twice as many cells in the test as control well was scored +; an even distribution of cells was scored 0; at least twice as many cells in control as test well was scored −. The scoring was done within 2 min of the beginning of the test.

A B C D E

FIGURE 3.—Apparatus for screening for chemokinesis-defective mutants. An exponential gradient of attractant to relative repellent in chamber C was separated from uniform concentrations of attractant (2.5 mM K$_2$ folate) in chamber A and relative repellent 5 mM KCl in chamber E by stopcocks B and D. Cells from a mutagenized population started in chamber A and all cells swimming down the gradient into relative repellent were collected from chamber E.

Genetic analyses

Crosses: Cells were crossed to d4-93, a recessive body-deformed mutant of 51-s, as described by Sonneborn (1970) and Kung (1971). Clones of the F$_2$ generation were isolated after the F$_1$ was induced to undergo autogamy. The F$_1$ and F$_2$ were scored for body morphology and behavior in well tests or T-mazes. The presence of the body deformation did not impair the cells from accumulating in attractant (data not shown; see Van Houten 1976).

Complementation tests: Folate mutants with body deformation markers were crossed with a second mutant with normal body morphology. The F$_1$ generation was scored for body and behavior. Some F$_2$ generations were isolated as a check on body deformation segregation.

RESULTS

Phenotypes

Mutants were tested for attraction to and dispersal from a series of normal attractants and repellents. The four representative mutants described (fol$^{1a}$, fol$^{1b}$, fol$^{2b}$ and fol$^{3}$), although abnormal in their population response to folate, were within the wild-type range in response to all other attractants and repellents tested in the T-maze (Figure 4). Normal cells are attracted to folate in the T-maze and well test assays whereas the mutants fol$^{1a}$, fol$^{1b}$ and fol$^{2}$ do not
accumulate in folate relative to chloride in either test (Figures 4 and 5). Mutant \textit{fol}^{2b} is not initially attracted to folate, as indicated in the rapid well test (Figure 5), but is eventually attracted in the T-maze assay (Figure 4). Time is apparently not limiting for responses of the other mutant lines.

**Genetic analysis of mutants**

All mutant lines were crossed to \textit{d4-93}, a behaviorally normal line with a recessive body deformation marker. The \(F_1\) generation of each cross was scored in well tests or T-mazes to determine if the mutations were dominant or recessive. As indicated in Table 1, all \(F_1\)s were wild type for behavior and morphology, indicating that all mutations were recessive. \(F_2\) clones of each cross were scored for attraction to folate relative to chloride and for body shape. As indicated in Table 1, all \(F_2\) generations segregate approximately 1:1:1:1 for normal body and behavior:normal body with fol\textsuperscript{−} behavior:body deformed with normal behavior:body deformed with fol\textsuperscript{−} behavior, as judged by a chi-square test at the 0.2 level of significance. These ratios indicate that each mutant harbored a genic, single site lesion that segregated in a Mendelian fashion, unlinked to the body-deformed locus.

Since all mutants were recessive, complementation tests could be performed to determine the number of loci represented by the mutants. Double fol\textsuperscript{−} behavior and body deformed mutants of each mating type were generated in
the F2 of crosses to d4-93. These double mutants were crossed to other fol−
mutants with normal body morphology and scored in the F1 for body and
behavior for complementation of the two fol− mutations (Table 2). Some double
body-deformed fol− mutants were crossed to their parental strains as comple-
mentation controls. Some complementation crosses were carried out to the F2
generation in order to detect linkage or suppression in double fol− mutants
(Table 3). The results in Tables 2 and 3 indicate that there are three comple-
mentation groups. Each group is discussed in turn below.

fol′a and fol′b are in complementation Group 1 (Table 2) and were selected by
two different procedures from separate mutagenized populations. There are
three other members of this group and none can be distinguished from other
group members by obvious differences in phenotype (Figures 4 and 5). No wild-
type recombinants are recovered from crosses of mutants within Group 1 (Table
3). However, sample sizes are relatively small and rare recombinants would be
M. C. Dinallo, M. Wohlford and J. Van Houten

TABLE 1

F₁ phenotypes and autogamous F₂ segregations between the fol⁻ mutants and d4-93

<table>
<thead>
<tr>
<th>Lines</th>
<th>F₁⁻</th>
<th>Normal</th>
<th>fol</th>
<th>Deformed</th>
<th>fol⁻ and de-</th>
</tr>
</thead>
<tbody>
<tr>
<td>fol¹ᵃ</td>
<td>Normal</td>
<td>11</td>
<td>11</td>
<td>11</td>
<td>14</td>
</tr>
<tr>
<td>fol¹ᵇ</td>
<td>Normal</td>
<td>22</td>
<td>21</td>
<td>24</td>
<td>30</td>
</tr>
<tr>
<td>fol¹ᶜ</td>
<td>Normal</td>
<td>25</td>
<td>21</td>
<td>20</td>
<td>23</td>
</tr>
<tr>
<td>fol¹ᵈ</td>
<td>Normal</td>
<td>11</td>
<td>11</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>fol¹ᵉ</td>
<td>Normal</td>
<td>8</td>
<td>13</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>fol²ᵃ</td>
<td>Normal</td>
<td>11</td>
<td>13</td>
<td>10</td>
<td>14</td>
</tr>
<tr>
<td>fol²ᵇ</td>
<td>Normal</td>
<td>14</td>
<td>11</td>
<td>6</td>
<td>17</td>
</tr>
<tr>
<td>fol²ᶜ</td>
<td>Normal</td>
<td>4</td>
<td>9</td>
<td>5</td>
<td>11</td>
</tr>
<tr>
<td>fol²ᵈ⁻</td>
<td>Normal</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>fol²ᵇ⁻</td>
<td>Normal</td>
<td>12</td>
<td>12</td>
<td>8</td>
<td>15</td>
</tr>
</tbody>
</table>

* F₁ phenotype scored in well test assay.
* Pass chi-square test at the 0.2 level of significance for expected 1:1:1:1 ratio of phenotypes for single-site mutations.
* F₂ generations were not isolated. Only F₁ phenotypes for dominance tests were scored.

TABLE 2

Complementation tests of fol⁻ mutants

<table>
<thead>
<tr>
<th>Line crossed to representatives of:</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>fol¹ᵇ</td>
<td>fol¹ᵈ</td>
<td>fol²ᵇ</td>
</tr>
<tr>
<td>Group 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>fol¹ᵃ</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>fol¹ᵇ</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>fol¹ᶜ</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>fol¹ᵈ</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Group 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>fol²ᵃ</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>fol²ᵇ</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>fol²ᶜ</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Group 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>fol²ᵇ⁻</td>
<td>(+)</td>
<td>(+*)</td>
<td>(+)</td>
</tr>
</tbody>
</table>

* F₁ is different from parents, but clearly not wild type. F₂ segregation ratios of this cross and fol²ᵇ × fol¹ᵇ (Table 3) indicate that both fol²ᵇ and fol¹ᵇ have mutations in genes separate from and unlinked to fol²ᵇ⁻. Parentheses indicate that same cross appears elsewhere in table.

missed. Crosses of mutants of Group 1 to mutants of other groups segregate in approximately 1:3 ratios (normal:fol⁻ phenotypes), as expected for unlinked mutants in different genes, with the exceptions of fol¹ᵇ and fol¹ᶜ crossed to fol²ᵈ⁻ (Table 3). In these crosses, more wild-type progeny appear in the F₂ generation than expected. The progeny fall into a 1:3 ratio that barely passes a chi-square test for a 1:3 ratio at the 0.2 level of significance. Since the preponderance of
wild-type phenotypes appears in crosses of two separate fol lines, it is possible that there is some suppression or other interaction between folzd and fol' gene products. However, the intergenic suppression is not complete because the crosses of folzd with fol' and fol'c fail to fit a 1:1 ratio at the level of 0.05 and 0.01, respectively. It is interesting to note, however, that cross of fol'c and folzd does fit an hypothesis of a third gene as suppressor of fol'c gene, a 3:5 (fol⁻ to normal) ratio. The F₂ passes a test of this ratio at the 0.99 level of significance. This suppressor would not explain the excess wild-type progeny in the cross of fol'c with folzd as the F₂ progeny of this cross fail to fit a 3:5 ratio at the level of 0.1-0.5 significance. This will be investigated further.

Group 2 is presently comprised of four lines of mutants (Table 2) that all originate from the same mutated population of cells and were isolated by the same Procedure 2 (see METHODS). Phenotypes within Group 2 are not identical, however. folza and folzd never show attraction to folate in well tests or T-mazes (Figures 4 and 5; Iche for folza = 0.46 ± 0.02). folzb and folzc are not attracted to folate in the well tests but, given sufficient time to respond, are eventually attracted in the T-maze (Figures 4 and 5; Iche for folzc = 0.64 ± 0.05). It is likely that folza and folzb are different alleles of the same gene folz and that folzd and folzc are copies of folza and folzb, respectively. Members of Group 2 show no intergenic interaction when crossed with mutants of other groups, except for folzd (discussed above) (Table 3). Members of Group 2 crossed with each other are expected to show no wild-type progeny. However, folza and folzb did yield 1 clone out of 82 that scored consistently as normal in the well test (Table 3). When the reciprocal cross was performed (Table 3) no wild-type progeny were detected, but the sample size was smaller than in the previous cross and rare
recombinants would have been missed. The wild-type F2 progeny was not likely
to result from an error in scoring and, therefore, may represent a recombinant
between fol2a and fol2b.

One mutant, fol1, comprises Group 3 (Table 2). fol1 was isolated from the same
population that included mutants of Groups 1 and 2. fol1 shows no strong
intergenic interaction when crossed with members of Groups 1 and 2 (Table 3).

**DISCUSSION AND CONCLUSIONS**

We have isolated ten lines of mutants that are not normal in response to
folate but are normal in all other chemokinesis responses tested. The time and
concentration of the tests are apparently not limiting except for fol2b and fol2c,
which will eventually accumulate in folate, given sufficient time in the T-maze.
These mutants are of the same complementation group as fol2a and fol2d that
are never attracted to folate.

The ten lines of mutants all harbor recessive, single site, genic mutations that
fall into three complementation groups. Crosses between either of two members
of complementation Group 1 and fol2d of Group 2 produce more wild-type F2
progeny than expected, but less than expected for full suppression interaction
between the genes fol1 and fol2. The crosses will be repeated and some of the
wild-type phenotype progeny analyzed to determine if they are double mutants,
true wild type, or heterozygotes because of unusual but possible macronuclear
regeneration after autogamy.

Members of Group 2 fall into two phenotype classes: a) never attracted to
folate (characteristic of fol2a and fol2d) and b) attracted in the T-maze at 30 min
(characteristic of fol2a and fol2c). Time course studies of responses in the T-maze
have confirmed that normal cells are immediately attracted to folate in the T-
maze; fol2a is never attracted; and fol2b begins to show slight attraction only
after 5 min (DiNallo, unpublished results). Crosses between fol2a and fol2b have
produced 1 clone of wild-type-phenotype out of 82 total progeny. The existence
of a wild-type recombinant would be consistent with the mutants fol2a and fol2b
being different alleles and producing different phenotypes. The percent recom-

bination (approximately 2.4%), however, would be higher than would be ex-
pected for intragenic recombination unless the gene were of considerable size.
More attempts will be made to isolate and analyze such rare recombinants.

The two methods of selection for fol- mutants were very different. Procedure
1 involved a slow enrichment procedure with retesting in the T-maze whereas
method 2 involved cloning of all cells swimming down a gradient of folate
attractant, similar to the method used to select d4-530, a mutant repelled by the
attractant sodium acetate (Van Houten 1976, 1977). All clones were retested in
Procedure 2, using the well test, a fast test of attraction or repulsion. The well
test measures the initial responses of cells to attractants and control solutions
and is comparable to tests run for only 2–5 min in the T-maze (Van HOUTEN,
Martel and Kasch, unpublished results). Cells could conceivably be unable to
adapt and sustain population responses for 30 min in the T-maze assay and yet
respond normally and quickly in the well test. Such abnormal cells would be
detected by Procedure 1 but overlooked by Procedure 2. [Such lines unable to
respond normally to ammonium have been isolated by Procedure 1 (Van
HOUTEN, Martel and Kasch, unpublished results).]
TABLE 4

Stocks, genotypes, phenotypes and genic symbols of fol− mutants of P. tetraurelia

<table>
<thead>
<tr>
<th>Stock</th>
<th>Genotype</th>
<th>Complementation group</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>d4-534</td>
<td>fol1a/fol1a</td>
<td>1</td>
<td>Note attracted to folate; otherwise appears normal</td>
</tr>
<tr>
<td>d4-535</td>
<td>fol1b/fol1b</td>
<td>1</td>
<td>Only mutant isolated by Procedure 1; similar to d4-534</td>
</tr>
<tr>
<td>d4-536</td>
<td>fol1a/fol1a</td>
<td>2</td>
<td>Not attracted to folate; otherwise appears normal</td>
</tr>
<tr>
<td>d4-537</td>
<td>fol1b/fol1b</td>
<td>2</td>
<td>Attracted to folate only if allowed to distribute more than 5 min in T-maze assay</td>
</tr>
<tr>
<td>d4-538</td>
<td>folb/folb</td>
<td>3</td>
<td>Only member of complementation Group 3; not attracted to folate; otherwise appears normal</td>
</tr>
</tbody>
</table>

Rescreening with the well test in Procedure 2 insured the isolation of mutants that were not capable of an initial response to attractant. The development of Procedure 2 stems from our interest in chemoreceptors. Mutants with defective receptors should be among those not able to respond even initially to folate. Mutants that require more time to accumulate in folate, although probably not having defective receptor function, would also be detected by Procedure 2 but overlooked by Procedure 1. Although all members of the complementation Group 2 have been isolated by the same Procedure 2, mutants fol2a and fol2d never respond to folate in wells or T-mazes, whereas fol2b and fol2c do not initially, but eventually, accumulate in the T-maze. These results demonstrate that Procedure 2 is successful at isolating mutants of either type of phenotype described above.

Two mutants of the same complementation Group 1 (fol1a and fol1b) were isolated by Procedures 1 and 2, respectively. Neither mutant is ever attracted to folate. Therefore, both procedures can be used to detect mutants of such phenotypes. Procedure 1 is less effective than Procedure 2, however, since fol1b is the only mutant fol− line isolated by this method.

It appears that eight of these mutant lines described possibly never detect the presence of folate in the external medium since they do not respond in the well test assay or in T-maze. If putative receptors for folate are defective in these mutants, the chemical signal will not be successfully transduced into the electrical signal in folate (VAN HOUTEN 1979) that in turn alters swimming behavior. The eight mutant cell lines successfully accumulate in or disperse from other compounds and give normal avoiding reactions in response to cations K, Ba and Na. Therefore, it is likely that the ciliary apparatus is normal and that some step in the chemosensory transduction pathway before the electrical signal arriving at the cilia is affected. We are presently examining binding and uptake of folate to determine if among these complementation groups are mutants defective in putative membrane chemoreceptors for folate.

Five lines of mutants are now assigned the standard d4 designation (derived stocks of P. tetraurelia). Table 4 summarizes these designations and mutant symbols.

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