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Phenylketonuric *Tetrahymena*: Phenylalanine hydroxylase mutants and other tyrosine auxotrophs

(tetrahydrobiopterin/dihydropteridine reductase/catecholamines/ciliapterin/serotonin)

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ABSTRACT **Nineteen tyrosine auxotrophs of the ciliated protozoan *Tetrahymena thermophila* have been isolated and biochemically examined. These mutants are defective in the conversion of phenylalanine to tyrosine; this is analogous to the defect that causes phenylketonuria in humans. After nitrosoguanidine mutagenesis and self-fertilization, progeny clones were screened for tyrosine auxotrophy and positively identified by using growth tests and *in vivo* radiometric assays for phenylalanine-to-tyrosine conversion. Mutants in one complementation group (locus) lacked phenylalanine hydroxylase activity; mutants in three other loci appeared to be deficient in the unconjugated pteridine cofactor that is necessary for the function of the hydroxylase. Another mutant lacked the dihydropterine reductase activity required to regenerate the reduced form of the pteridine cofactor. Because hydroxylation of tyrosine to dopa and of tryptophan to 5-hydroxytryptophan may require the same cofactor and pterin reductase as phenylalanine hydroxylase, these mutants may also prove useful for the study of the role of catecholamines and serotonin, substances known to be present in *Tetrahymena*.**

Phenylketonuria is a hereditary human disease caused by a deficiency in the enzymatic conversion of phenylalanine to tyrosine. This deficiency can result from a lack of any one of at least three components: phenylalanine hydroxylase, which directly converts phenylalanine to tyrosine (1–3); tetrahydrobiopterin, which acts as a hydrogen donor in the hydroxylation (4); and dihydropteridine reductase, which catalyzes the conversion of oxidized dihydrobiopterin to its active tetrahydro form (5). The biochemical and clinical aspects of this disease have been extensively studied (6, 7).

The ciliated protozoan *Tetrahymena* is a useful, animal-like model system for the isolation and study of tyrosine auxotrophs. Recently developed methods for efficient isolation of recessive mutants in *Tetrahymena* (ref. 8; unpublished data) make possible the isolation of biochemical mutants. The ease of biochemical and genetic manipulation of this organism allows a quick and detailed analysis of the mutation in question. The nutritional requirements for *Tetrahymena* have been worked out in detail, yielding both a defined medium for growing the cells (9) and useful information for hypothesizing about metabolic pathways in this organism.

Tetrahymena and the growing rat have identical amino acid requirements (9, 10). For both organisms, the phenylalanine requirement is approximately halved when the nonessential amino acid tyrosine is included in the diet (9, 11). Also, at low enough concentrations of phenylalanine, growth in *Tetrahymena* becomes dependent on tyrosine supplementation (12). These clues suggested that the conversion of phenylalanine to tyrosine in this ciliate resembles the mammalian pathway.

In the present paper, we report the isolation of tyrosine auxotrophs in *Tetrahymena thermophila*; as such, these mutants may be considered to be "phenylketonuric." Our biochemical and genetic complementation studies indicate that at least seven different kinds of defects can block the phenylalanine-to-tyrosine conversion in this organism. Several mutations, all in the same locus, cause the lack of phenylalanine hydroxylase activity. Another mutant lacks dihydropteridine reductase. The growth of other mutants at several different loci is stimulated by 6-methyltetrahydropterine, and they are probably blocked in the unconjugated pteridine biosynthetic pathway.

MATERIALS AND METHODS

Strains. Cells of inbred strain B *T. thermophila* [formerly *T. pyriformis*, syngen 1 (13)] were used in all experiments. Stock maintenance and other routine procedures have been described in detail (8, 14, 15).

Media. A modification (16) of the chemically defined medium of Holz *et al.* (17) was primarily used to test auxotrophy; when necessary, defined medium was supplemented with 1.1 mM tyrosine (defined medium/Tyr). Two complex media were used for routine growth of cultures: PP210, consisting of 2% proteose peptone (Difco) supplemented with 10 μM ferric chloride; and PP110.15, consisting of 1% proteose peptone supplemented with 10 μM ferric chloride and 0.15% yeast extract (Difco). Proteose peptone media contain sufficient tyrosine to allow for normal growth of the auxotrophs. Penicillin G and streptomycin sulfate (Sigma), each at 250 μg/ml, were added to all the above nutrient media to help prevent bacterial contamination. Unless otherwise indicated, cells were grown in 10–20 ml of medium without shaking, in 100 × 15 mm sterile disposable Petri dishes (Plasta-Medic).

Cells were washed and assayed in 10 mM or 50 mM Tris·HCl at pH 7.5 (Sigma) (15).

Mutant Isolation. SB210 cells were exposed to nitrosoguanidine and crossed to CU329 cells. Self-fertilization (cytogamy) was induced in most of the pairs by giving a hyperosmotic shock with 1.5% glucose at 5 hr after the starved cells were mixed (18). Self-fertilized progeny derive their nuclear genetic information from a single haploid meiotic product and hence become homozygous at every locus (14). Progeny were cloned in 96-well plates under conditions that select for self-fertilized progeny, exploiting the recessiveness of the 2-deoxygalactose-resistance mutation (19). The cloned progeny were then replicated to PP210 (or PP110.15) and defined medium at 38°C, and those that grew poorly or not at all in defined medium but well in complex medium, were then tested in defined medium + Tyr.

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Abbreviation: $MePteH_4$, 6-methyl-5,6,7,8-tetrahydropterin.

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The presumptive tyrosine auxotrophs were then phenotypically retested twice before further analysis. Nineteen mutants were isolated in this fashion.

The first two mutants were isolated by treating CU324 with a mutagen, using short-circuit genomic exclusion (8) instead of cytogamy, and selecting self-fertilized progeny with 6-methylpurine. These progeny were replicated to defined medium and defined medium lacking the free amino acids but supplemented with 0.1% coagulated egg albumin [as a complete amino acid source (20)] in the initial mutant screening. Clones that grew in defined medium plus egg albumin but not in defined medium were then tested specifically for tyrosine auxotrophy in defined medium + Tyr.

Growth Rate Measurements. Cells freshly grown in PP210 or PP110.15 were inoculated into defined medium and allowed to grow for 1 day to deplete internal tyrosine pools and any tyrosine carried over with the inoculum. Each clone in defined medium culture was then inoculated at 5000 cells per ml into fresh defined medium and defined medium + Tyr at 30°C and 38°C. Cell concentrations were determined at various times by using a Celloscope cell counter.

***In Vivo* Radiometric Assay for Phenylalanine-to-Tyrosine Conversion.** This assay is an adaptation of Milstien and Kaufman's protocol (21). All procedures were carried out in an ice bath unless otherwise indicated. Cells were grown in defined medium + Tyr at 30°C to late logarithmic phase, washed twice in 10 mM Tris buffer, and resuspended at a ratio of 1:1 (vol/vol). The reaction mixture included 2 mM L-phenylalanine, 9.4 μM L-[U-^{14}C]phenylalanine (Amersham; 2.4 mCi/mmol; 1 Ci = 3.7×10^{10} becquerels), 6.6 mM Tris, cycloheximide at 15 μg/ml (to inhibit incorporation of label into protein), and freshly washed cells at 2.0–8.0 mg of cell protein per ml. The usual reaction volume was 50 μl. All ingredients except the cells were mixed in the reaction tube (Sarstedt 72.690), and the cells were spun down and added just prior to incubation, which was at 30°C or 38°C for 90 min.

The reaction was stopped with 3 vol of 12% (wt/vol) trichloroacetic acid, and the precipitated proteins were sedimented at 18,000 × *g* (Sorvall SS-34). Unlabeled phenylalanine and tyrosine (20 nmol of each) in 10 μl were spotted as carriers at the origin on a chromatographic plate (Analtech MN300 cellulose, normal, 2.5 × 10 cm) followed by 10 μl of reaction supernatant. The chromatogram was developed in $CHCl_3/CH_3OH/NH_4OH/H_2O$, 5.8:3.2:0.8:0.2 (vol/vol); phenylalanine R_F = 0.6, tyrosine R_F = 0.2. After air drying, the chromatogram was sprayed with ninhydrin and warmed until amino acid bands appeared. The origin and tyrosine and phenylalanine bands were scraped into separate glass scintillation vials and moistened with 0.5 ml of H_2O. After addition of 5 ml of Aquasol (New England Nuclear), each vial was assayed in a liquid scintillation counter (Beckman LS-230). Protein content of the cells was determined by the Bradford method (22) (Bio-Rad).

Data were expressed as nmol of tyrosine formed per mg of cell protein during the 90-min reaction (after subtraction of background tyrosine counts, around 155 cpm, determined in control reactions using boiled or trichloroacetic acid-precipitated cells). Conversion in wild-type cells was linear with time and amount of extract under the conditions of the assay.

A significant amount of radioactivity was also found at the origin of the chromatogram, but only in those cases in which a considerable quantity of tyrosine was produced (wild type, origin cpm = 2.8%; origin mutants 2 and 4, origin cpm = 0.6%. From this correlation, we assume that these values represent tyrosine metabolites, but no attempt has been made to characterize them further. No significant radioactivity above background was found anywhere else in the chromatogram.

RESULTS

Isolation of Tyrosine Auxotrophs. Twenty-one tyrosine auxotrophs have been isolated; three of these failed to produce sexual progeny and have not been studied further. The mutant frequency is 1.5×10^{-4} viable excytogamous progeny when treated according to our protocol and 10^{-3} for two mutants obtained according to Bruns and Sanford (8).

We partially characterized the mutants with respect to the following properties: doubling time; *in vivo* conversion of phenylalanine to tyrosine; growth stimulation by ascorbate, 6-methyl-5,6,7,8-tetrahydropterin ($MePteH_4$) (Calbiochem) + ascorbate, Fe^{2+}, Fe^{3+}, and high phenylalanine; and *in vitro* phenylalanine hydroxylase activity (Table 1). Some of these properties are treated in more detail below.

All the mutants tested were restored to a wild-type growth rate by tyrosine addition. A few of the mutants were leaky at 30°C but tighter at 37°C. Radiometric assay results for *in vivo* conversion of phenylalanine to tyrosine were consistent with the auxotrophy revealed by growth curves and confirm the idea that phenylalanine is a precursor of tyrosine biosynthesis in *Tetrahymena*.

Biochemical Characterization. An *in vitro* assay specific for phenylalanine hydroxylase (23) was adapted for wild-type *Tetrahymena* cells. Excess reduced pteridine cofactor in the form of $MePteH_4$ and reducing power in the form of ascorbate were used to bypass the need for endogenous natural cofactor and reductase (24). The reaction clearly was dependent on the presence of the pteridine cofactor and somewhat independent of components that in a purified rat liver system, play a role in the regeneration of the tetrahydro cofactor (NADP, NADPH, NAD, or NADH and its regenerating system glucose dehydrogenase and glucose; or chemical reduction by ascorbate) (Table 2). Fairly high concentrations of $MePteH_4$ (4 mM) were required for optimal conversion of phenylalanine to tyrosine, even under such reducing conditions. The latter two observations indicate that the cofactor may not be recycling to any great extent in our crude system. $MePteH_4$ was a much more effective cofactor for *Tetrahymena* phenylalanine hydroxylase than was 6,7-dimethyl-5,6,7,8-tetrahydropterin (Calbiochem). No significant difference in activity was observed when NADPH, NAD, or NADH replaced NADP or upon addition of catalase or protease inhibitors to the assay (unpublished data). The substrate L-phenylalanine significantly protected the enzyme upon cell disruption (Table 2).

Results of phenylalanine hydroxylase assays for each of the tyrosine auxotrophs are shown in Table 1. All four mutants in the *tyrC* locus had little if any phenylalanine hydroxylase activity; the other mutants had levels close to or higher than wild-type levels. The wild-type hydroxylase showed full activity in the presence of mutant C3 extract; thus, this auxotroph does not contain an inhibitory substance but rather lacks a functional phenylalanine hydroxylase (unpublished data). Mutant *tyrC16* was stimulated *in vivo* by high concentrations of phenylalanine (18 mM), and may well be a "K_m" mutant. Two sterile mutants (not shown) also lacked phenylalanine hydroxylase activity. Mixture of extracts of one of these with mutant C3 extracts failed to restore activity. Unpublished genetic evidence suggests that at least two mutant sites are represented among the four fertile mutants. We suggest that *tyrC* is the structural (rather than a regulatory) gene for the phenylalanine hydroxylase because, among the large variety of mutants and loci already identified, mutants at only that locus lack phenylalanine hydroxylase activity.

The increases in phenylalanine hydroxylase activity seen in most of the other mutants were slight but reproducible and may

Table 1. Physiological and biochemical properties of tyrosine auxotrophs

Mutants* and loci	Doubling time, hr†		*In vivo* Phe-to-Tyr conversion,‡ %	Growth stimulation§				*In vitro* Phe hydroxylase activity,¶ %
	Defined medium	Defined medium +Tyr		$MePteH_4$ + Asc	Asc	Fe^{2+}	Fe^{3+}	
tyr+	6.5	6.5	100					100
tyrA1	1.0	6.5	5	−	−	−	−	158
tyrA5	<1.0	6.8	<5	−	−	−	−	127
tyrA11				−	−	−	−	111
tyrB2	<1.0	6.4	22	+	(−)	+	(+)	108
tyrB4	<1.0	6.8	29	+	−	+	−	115
tyrC3	<1.0	6.8	<5	−	−	−	−	<5
tyrC12				−	−	−	−	13.5
tyrC16				−	−	−	−	<5
tyrC17				−	−	−	−	<5
tyrD6‖	1.2	6.3	8	(+)	−	(+)	−	135
tyrE10				−	−	−	−	95
tyrF13				+	(+)	−	−	68
tyrG15‖				+	+	+	+	166
tyr-7	2.2	7.1	5	(+)	−	−	−	138
tyr-9‖				−	−	−	−	66
tyr-14‖				−	−	−	−	93
tyr-18				(+)	(+)	−	−	126
tyr-19				−	−	−	−	114

* Loci (= complementation groups) *tyrA–tyrE* were assigned on the basis of the phenotype of double heterozygotes. Mutants 13 and 15 were assigned to loci *F* and *G* on the basis of chromosome location (mutant 13) and lack of dihydropteridine reductase activity (mutant 15). Mutants with a hyphenated name have not yet been assigned to a locus but complement with groups *A–D* (unpublished data).

† After 31 hr at 30°C (or 25 hr at 38°C).

‡ Wild-type activity was defined as 100% for each experiment; it varied between 23 and 30 nmol/mg per 90 min at 30°C and was 46 nmol/mg per 90 min at 38°C. Percentages are derived from three separate experiments and represent averages of at least two reaction mixtures per mutant.

§ Compounds were added to defined medium (without tyrosine), each at 1 mM (Asc, ascorbate). Tests were done in 96-well plates at 37°C. Responses: +, strong; (+), intermediate; (−), slight; −, none.

¶ Sonication and assay conditions as in Table 2. Wild-type activity was defined as 100% for each experiment; it varied between 0.23 and 0.31 unit of enzyme activity per mg of protein at 30°C and was 0.21 unit/mg at 38°C; four experiments are represented. Each percentage represents an average of at least two reactions. For mutants *A1*, *B2*, *C3*, *B4*, *A5*, and *D6*, two F_2 homozygous progeny were also tested; mutant and progeny results were essentially identical in every case except for mutant 1. (F_2 progeny of mutant 1 are peculiar in that they show decreased hydroxylase activity: 81% of wild type compared to 158% for the original mutant. The F_2 progeny also grow poorly in PP210 medium unless it is supplemented with yeast. The reasons for these two peculiarities, and whether or not they are biochemically related, are unknown.)

‖ Partially temperature-sensitive mutants; assays were performed at 37°C.

have a regulatory basis. However, the variation of tyrosine and phenylalanine concentrations in the growth medium did not cause any significant changes (>1.5-fold) in phenylalanine hydroxylase activity (unpublished data).

Assays of our mutants for dihydropteridine reductase were kindly performed by S. Milstien and S. Kaufman (Laboratory of Neurochemistry, National Institute of Mental Health). The assay (25) measures the NADH-dependent reduction of "quinonoid" 6,7-dimethyldihydropterin to the tetrahydro form. Tyrosine auxotroph 15 had greatly decreased levels of reductase; all the other mutants showed levels close to wild type. Mutant 15 responded well *in vivo* to the addition of ascorbate or increased levels of iron salts (Table 1).

Because human tyrosine auxotrophs can be deficient in pteridine cofactor biosynthesis (5), we screened our mutants for growth stimulation (in the absence of tyrosine) by $MePteH_4$, a relatively stable analog of the natural cofactor (26). Mutants in at least three different loci (*B*, *D*, and *F*) showed varying (but nevertheless significant and repeatable) degrees of stimulation (Tables 1 and 3). *B* mutants also responded to biopterin (in the presence of ascorbate) but less well (Table 3 and unpublished data; other mutants not tested). It seems likely that at least some of these mutants are blocked in the unconjugated pteridine biosynthetic pathway and thus are primarily pteridine auxotrophs.

Mutants *B2* and *B4* also were stimulated by the addition of Fe^{2+} or (less well) Fe^{3+} (Table 1). Other divalent cations (Cu^{2+}, Ca^{2+}, Mg^{2+}) had no effect (unpublished data). These two mutants are also interesting in that they exhibited little, if any, growth in defined medium and yet were able to produce a sizable amount of tyrosine (Table 1). It is not clear if this is a real paradox or merely the result of the different recent history of the cells used for growth and phenylalanine conversion assays or the differences in time scale of the experiments (S. Kaufman, personal communication). Tests for stimulation of the other mutants by Fe salts revealed a number of other responders and a partial correlation with the ability to respond to $MePteH_4$.

With the exception of mutants *C16* (referred to above) and *D6* (which showed a slight but inconsistent stimulation), none of the other mutants in Table 1 showed growth stimulation by 18 mM phenylalanine in defined medium (10-fold higher than normal) in the absence of tyrosine. This suggests that few (if any) of our mutants are "pseudo-tyrosine-auxotrophs" due to a primary defect in phenylalanine uptake.

DISCUSSION

Nineteen tyrosine auxotrophs of the unicellular eukaryote *T. thermophila*, distributed among at least seven complementa-

Table 2. Phenylalanine hydroxylase assay: dependence on various components of the reaction and sonication mixtures

Component omitted	Mixture omitted from	Activity, %
None		100
Cell extract (sonicate)	Reaction	<5
$MePteH_4$	Reaction	<5
L-Phenylalanine	Sonication/reaction	<5
L-Phenylalanine	Sonication	21
Ascorbate	Sonication/reaction	61
Ascorbate	Sonication	80
Glucose dehydrogenase, glucose, and NADP	Reaction	82

All procedures were carried out in an ice bath unless otherwise indicated. Wild-type cells were grown in defined medium and washed as described under *Materials and Methods*, except 10 mM Tris was replaced with 50 mM. The 400-μl sonication mixture typically contained 0.166 M sodium ascorbate at pH 7.0, 15.5 mM L-phenylalanine, 25 mM Tris, and freshly washed cells at 7.5–17.5 mg of cell protein per ml. The cells were then sonicated at 50 W for 30 sec with a microtip (Sonifier cell disruptor W185D, Heat Systems-Ultrasonics, Inc.). The complete reaction mixture contained, typically in a total volume of 50 μl, 3.1 mM L-phenylalanine, 9.4–18.8 μM L-[U-^{14}C]phenylalanine (final, 1.56–3.12 mCi/mmol), 0.175 unit of glucose dehydrogenase (Sigma), 0.24 M glucose, 1.25 mM NADP (Sigma), 10 μl of the above sonicate, and 4 mM $MePteH_4$. The pteridine was prepared and added just prior to incubation, which was at 30°C for 30 min. The reaction was then stopped with 3 vol of 12% trichloroacetic acid. The phenylalanine hydroxylase activity of the complete reaction mixture (100%), averaged from the three experiments used to compose the table, was 0.29 unit/mg of protein [1 unit equals the amount of enzyme that catalyzes the formation of 0.1 μmol of tyrosine in 30 min (24)]. Other percentages represent averages of at least two reactions. Only the tyrosine and phenylalanine regions of the chromatogram contained radioactivity significantly higher than background; at least 89% of the total radioactivity spotted was generally recovered in these two bands. Given the range of experimental error, 5% is probably the limit of sensitivity of the assay. Conversion was linear with time and amount of extract under the assay conditions.

tion groups, have been obtained. Mutations in one locus (*tyrC*) cause the loss of phenylalanine hydroxylase activity. A mutation in another locus (*tyrG*, tentatively) results in little or no dihydropteridine reductase. Effects of mutations in at least three loci (*tyrB*, *-D*, and *-F*, respectively) are phenotypically reversed (at least partially) by addition of a tetrahydropterin to the growth medium; at least some of those mutants may be blocked in unconjugated pteridine biosynthesis. The meaning of the growth response of some of these mutants to iron salts remains to be elucidated.

The rat liver system has been invaluable as a model in this investigation of the conversion of phenylalanine to tyrosine in *Tetrahymena*. Several observations made here suggest a strong parallel between the two systems. First, several loci have been identified in both systems, indicative of the biochemical complexity that surrounds, more or less specifically, the hydroxylation of phenylalanine. Second, a phenylalanine hydroxylase has been identified in *Tetrahymena* with activity comparable to that of crude liver extracts. By using Kaufman and Levenberg's data for rat liver enzyme (phenylalanine hydroxylase) response to $MePteH_4$ (26) and assuming a 59-fold purification of this enzyme (23), tyrosine yield from a crude extract should be approximately 76 nmol/mg of protein per 30 min, whereas the *Tetrahymena* yield from under similar conditions is 29 nmol.

A third analogy between *Tetrahymena* and mammalian liver is the participation of a reduced unconjugated pteridine cofactor, as indicated by the $MePteH_4$ requirement in the phenylalanine hydroxylase assay (Table 2), the growth response of mutants affected in several loci to $MePteH_4$ (Tables 1 and 3),

Table 3. Growth responses (number of doublings) of certain tyrosine auxotrophs to pteridines

	Doublings, no./72 hr			
		Mutant strains		
Medium	Wild type	B2	B4	C3
Defined medium	6.6	<1	<1	<1
Defined medium + Tyr	7.0	6.4	6.1	6.7
Defined medium + ascorbate	6.4	1.7	1.8	<1
Defined medium + $MePteH_4$	6.3	2.8	1.7	<1
Defined medium + $MePteH_4$ + ascorbate	6.0	4.7	3.8	<1
Defined medium + biopterin	6.2	<1	<1	<1
Defined medium + biopterin + ascorbate	5.8	3.5	2.0	<1

Cultures were grown at 30°C in 17 × 100 mm sterile plastic disposable tubes (Falcon). Ascorbate, $MePteH_4$, and biopterin were added to final concentrations of 1 mM.

and the lack of dihydropteridine reductase in one tyrosine auxotroph (S. Milstien and S. Kaufman, personal communication). Kidder and Dewey (27) have shown that *Tetrahymena* synthesizes a compound that fulfills the unconjugated pteridine requirement for *Crithidia fasciculata* (28). This pteridine, named "ciliapterin," appears to differ from the mammalian cofactor only in its side chain configuration [L-*threo* instead of L-*erythro* (27)]. Barak (29) concluded that, in *T. pyriformis*, ciliapterin is the cofactor of choice for both the phenylalanine and tyrosine hydroxylases, with a dihydropteridine reductase converting dihydrociliapterin to its active tetrahydro form. Experiments with our pteridine-responding mutants may prove useful for the further investigation of *Tetrahymena*'s natural hydroxylation cofactor and its corresponding reductase. These mutants may also prove useful for elucidating the unconjugated pteridine biosynthetic pathway.

Of additional interest is the fact that *Tetrahymena* has a catecholamine synthesis pathway (30) resembling the mammalian pathway in that it also starts from tyrosine, although the detailed correspondence has been questioned (31). Because in mammalian cells the hydroxylation of tyrosine (in the catecholamine pathway) and tryptophan (in the serotonin pathway) requires the same cofactor and reductase as the hydroxylation of phenylalanine, it seems quite likely that some of the mutants described here may be deficient also in the synthesis of these compounds.

The role of catecholamines in *Tetrahymena* has not been well described, although it is likely that these cells possess an adrenergic metabolic control system (32). Catecholamines have been implicated in *Tetrahymena* carbohydrate metabolism (32) and, more specifically, in galactokinase regulation (33). Some of the tyrosine auxotrophs isolated may be useful for experimental manipulation of catecholamine levels in *Tetrahymena* and might help to elucidate general aspects of the synthesis, metabolism, function, regulation, and dispensability of these messengers. The ease of biochemical manipulation of *Tetrahymena* makes it ideally suited for such a study.

The technology for efficiently isolating and examining more tyrosine as well as other types of auxotrophs is now available. Other laboratories have already isolated proline (34) and unsaturated fatty acid (35) auxotrophs, but these have not been characterized genetically. We have isolated several proline, cysteine, and thymidine auxotrophs from the same population of mutagen-treated progeny as the tyrosine auxotrophs; these have not yet been investigated.

Although the biochemical and clinical aspects of phenylketonuria have been extensively studied (6), further examination

of these "phenylketonuric" *Tetrahymena* seems worthwhile. Primarily, this ciliate system allows for a quick and easy genetic dissection of the reaction, perhaps yielding relevant information about the hereditary aspects of this complex disease. These cells, which grow rapidly (2.2-hr generation time) to a high density (10^6/ml) in complex medium, are a potential source of inexpensive extracts and compounds which may be useful in diagnosing or treating phenylketonuria. Finally, because these eukaryotes are so amenable to biochemical analysis, they may help to determine whether natural unconjugated pteridines, dihydropteridine reductase, or other phenylalanine hydroxylation components have other metabolic roles in the cell. For example, pteridines appear to be involved in the biosynthesis of unsaturated fatty acids, sterols, and pyrimidines in the flagellate *Crithidia fasciculata* (36). If biopterin-deficient phenylketonurics have similar metabolic defects, such knowledge may be instrumental in designing the most effective clinical treatment.

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1. Wallace, H. W., Moldave, K. & Meister, A. (1957) *Proc. Soc. Exp. Biol. Med.* **94**, 632–633.
2. Mitoma, C., Auld, R. M. & Udenfriend, S. (1957) *Proc. Soc. Exp. Biol. Med.* **94**, 634–635.
3. Kaufman, S. (1958) *Science* **128**, 1506–1508.
4. Kaufman, S., Berlow, S., Summer, G. K., Milstien, S., Schulman, J. D., Orloff, S., Spielberg, S. & Pueschel, A. (1978) *N. Engl. J. Med.* **299**, 673–679.
5. Kaufman, S., Holtzman, N. A., Milstien, S., Butler, I. J. & Krumholz, A. (1975) *N. Engl. J. Med.* **293**, 785–790.
6. Kaufman, S. & Milstien, S. (1977) *Ann. Clin. Lab. Sci.* **7**, 178–185.
7. Kaufman, S. (1976) in *Advances in Neurochemistry*, ed. Agranoff, B. W. & Aprison, M. H. (Plenum, New York), Vol. 2, pp. 1–132.
8. Bruns, P. J. & Sanford, Y. M. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 3355–3358.
9. Kidder, G. W. & Dewey, V. C. (1951) in *Biochemistry and Physiology of Protozoa*, ed. Lwoff, A. (Academic, New York), Vol. 1, pp. 323–400.
10. Rose, W. C., Oesterling, M. J. & Womack, M. (1948) *J. Biol. Chem.* **176**, 753–762.
11. Womack, M. & Rose, W. C. (1946) *J. Biol. Chem.* **166**, 429–434.
12. Zdanowski, M. K. & Rasmussen, L. (1979) *Comp. Biochem. Physiol. A* **62**, 331–334.
13. Nanney, D. L. & McCoy, J. W. (1976) *Trans. Am. Microsc. Soc.* **95**, 664–682.
14. Orias, E. & Hamilton, E. P. (1979) *Genetics* **91**, 657–671.
15. Orias, E. & Bruns, P. J. (1976) in *Methods in Cell Biology*, ed. Prescott, D. M. (Academic, New York), Vol. 13, pp. 247–282.
16. Rasmussen, L. & Modeweg-Hansen, L. (1973) *J. Cell Sci.* **12**, 275–286.
17. Holz, G. G., Jr., Erwin, J., Rosenbaum, N. & Aaronson, S. (1962) *Arch. Biochem. Biophys.* **98**, 312–322.
18. Orias, E., Hamilton, E. P. & Flacks, M. (1979) *Science* **203**, 660–663.
19. Roberts, C. T. & Morse, D. E. (1980) *Mol. Gen. Genet.* **180**, 129–134.
20. Orias, E. & Rasmussen, L. (1979) *J. Cell Sci.* **36**, 343–353.
21. Milstien, S. & Kaufman, S. (1975) *J. Biol. Chem.* **250**, 4777–4781.
22. Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248–254.
23. Kaufman, S. (1962) *Methods Enzymol.* **5**, 809–816.
24. Kaufman, S. (1959) *J. Biol. Chem.* **234**, 2677–2682.
25. Craine, J. E., Hall, E. S. & Kaufman, S. (1972) *J. Biol. Chem.* **247**, 6082–6091.
26. Kaufman, S. & Levenberg, B. (1959) *J. Biol. Chem.* **234**, 2683–2688.
27. Kidder, G. W. & Dewey, V. C. (1968) *J. Biol. Chem.* **243**, 826–833.
28. Nathan, H. A., Hutner, S. H. & Levin, H. L. (1958) *J. Protozool.* **5**, 134–138.
29. Barak, A. (1973) Dissertation (Tel-Aviv Univ.) (English abstr.).
30. Janakidevi, K., Dewey, V. C. & Kidder, G. W. (1966) *J. Biol. Chem.* **241**, 2576–2578.
31. Kaney, A. R. & Knox, G. W. (1980) *J. Protozool.* **27**, 339–341.
32. Blum, J. J. (1967) *Proc. Natl. Acad. Sci. USA* **58**, 81–88.
33. Roberts, C. T. & Morse, D. E. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 1810–1814.
34. Hepfer, C. E. & Kaney, A. R. (1976) *J. Protozool.* **23**, 22A (abstr.).
35. Hill, R. J. (1980) *Biochim. Biophys. Acta* **595**, 140–145.
36. Kidder, G. W. & Dewey, V. C. (1963) *Biochem. Biophys. Res. Commun.* **12**, 280–283.