agent, since concentrations of MPTP in the tissue are tenfold higher in pargyline-protected animals than in animals not treated, after each group received toxic amounts of MPTP. Pargyline has also been shown to prevent the dopamine depleting effect of MPTP in mouse striatum (*9*).

The studies reported here also provide evidence for a possible molecular mechanism of action for MPTP. We have shown earlier that removing the double bond at the "4-5" position in MPTP blocks the conversion of MPTP to MPP^+ (*10*) and the toxic effects of the compound. While it is possible that pargyline prevents MPTP neurotoxicity by some means other than preventing the conversion of MPTP to MPP^+ (presumably by blocking MAO activity), we have now demonstrated by two different approaches that blocking this conversion parallels prevention of neurotoxicity. Pargyline actually appears more effective in blocking this conversion in the central nervous system, raising the possibility of a differential effect in the central nervous system as compared to systemic tissues. Our results still do not explain why MPTP is selectively toxic to the substantia nigra, although we suspect that this is in some way related to the high dopamine concentrations in the nigrostriatal system.

Another MAO inhibitor deprenyl (selective for MAO-B) has been used for a number of years as an adjunct in the therapy of Parkinson's disease (*11*). In that deprenyl may be effective in retarding the progress of Parkinson's disease (*12*), and in that MAO inhibitors prevent MPTP-induced parkinsonism, it seems that testing the early use of deprenyl or other MAO inhibitors to alter the course of the disease may be warranted.

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δ-Aminolevulinic Acid–Synthesizing Enzymes Need an RNA Moiety for Activity

Abstract. *When* Chlamydomonas *enzymes that convert glutamate to δ-aminolevulinic acid are separated into three fractions, one of the fractions contains RNA, and the RNA moiety is needed for the enzyme activity.*

δ-Aminolevulinic acid (ALA) is a rate-limiting factor of the chlorophyll biosynthetic pathway. It is synthesized from glutamate in plastids and is the first committed intermediate of the chlorophyll biosynthetic pathway (*1*). The enzymes from barley plastids (*2*) and *Chlamydomonas* cells (*3*) that are responsible for the formation of ALA from glutamate have been isolated and fractionated into three parts by serial affinity chromatography with a Blue Sepharose column and a heme-Sepharose column. The fractions are designated as Blue Sepharose–bound (B), heme-Sepharose–bound (H), and runoff (R). When combined together, the fractions can convert glutamate to ALA, but they are inactive when assayed alone. Cofactors required are adenosine triphosphate (ATP), Mg^{2+}, and the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) (*2, 3*).

The best supported scheme for ALA formation from glutamate is shown in Fig. 1. It had been proposed that glutamate is activated by a kinase using ATP and Mg^{2+} to form glutamate-1-phosphate. Subsequently, a dehydrogenase converts glutamate-1-phosphate to glutamate-1-semialdehyde (GSA). Finally, GSA is converted to ALA (*4*). GSA has been synthesized from *N*-carbobenzoxyglutamatic acid–γ-benzyl ester (*4*), and the structure of its acetal has been verified (*5*). The enzyme GSA aminotransferase (E.C. 5.4.3.8), which converts GSA to ALA, has been purified from barley plastids (*6*). Since none of the other intermediates have been isolated and identified, the exact nature of the proposed reaction sequence remains unclear. However, we have shown that (i) GSA aminotransferase is in the R fraction (*2, 3*); (ii) the H and B fractions together convert glutamate to a compound that purifies with synthesized GSA on high-performance liquid chromatography (*2*); and (iii) the compound synthesized by the H and B fractions from glutamate, as well as the synthesized GSA, can be converted by the R fraction to ALA (*2, 3*). This ALA was identified by high-performance liquid chromatography and thin-layer chromatography against authentic samples of ALA.

Under the assumption that H and B

Table 1. Effects of ribonuclease and deoxyribonuclease on the enzyme activity of crude enzyme preparation and H + B fractions. The assay of ALA-synthesizing activity and the method of purifying the product are as described (*2*). The product of the H + B reaction mixture is glutamate-1-semialdehyde. It can be quantitatively recovered by purification procedure used. For more detail, see text. Activity is given as counts per minute per milligram of protein per hour.

Sample	Activity ($cpm \cdot mg^{-1} \cdot hour^{-1}$)
Crude enzyme	100,580
Crude enzyme + ribonuclease*	2,169
Crude enzyme + ribonuclease + ribonucleasin†	93,703
H + B	15,327
H + B + ribonuclease	4,648
H + B + ribonuclease + ribonucleasin	16,076
H + B + deoxyribonuclease‡	13,538
H + B + deoxyribonuclease + ribonucleasin	17,636

*Ribonuclease A (Sigma), 1 μg per milliliter of enzyme assay. †Ribonucleasin (Promega Biotec), 500 units per milliliter of enzyme assay. ‡Deoxyribonuclease I, highest purity (Worthington Diagnostic), 2 μg per milliliter of enzyme assay.

fractions together can convert glutamate to GSA, we have been studying the components of the ALA-synthesizing enzymes in either H or B fractions by the following assay. We combine the H fraction with the B fraction and add radioactive glutamate plus all the cofactors. After incubation, radioactive GSA is purified, and the radioactive label is counted (*3*). Since we have been able to recover GSA quantitatively with our purification procedure (*2, 3*), this is considered a reliable and justifiable assay for ALA-synthesizing-enzyme components in H and B fractions.

The heme-bound fraction, H, has high absorbance at 260 nm in comparison with the B or R fractions (Fig. 2A). When the H fraction was treated with 40 μl of 70 percent cold $HClO_4$ and the precipitate was removed by centrifugation, the absorbance at 260 nm was no longer detectable in the supernatant. The absorbance at 260 nm remained in the supernatant if the H fraction was treated with ribonuclease before acid precipitation (Fig. 2B). However, if placental ribonucleasin, a specific inhibitor of ribonuclease, was added to the H fraction together with the ribonuclease, no absorption at 260 nm was detectable in the supernatant after acid precipitation (Fig. 2B). We concluded that the high 260-nm absorbance in the H fraction was due to the presence of RNA.

Next, the H fraction was analyzed by polyacrylamide gel electrophoresis without sodium dodecyl sulfate (SDS). After silver-staining, we observed a few bands that migrated much faster and to the same positions as the RNA control [transfer RNA (tRNA) from yeast] (Fig. 3). Unlike the more slowly migrating protein bands, these bands could not be stained by Coomassie blue but could be stained by ethidium bromide and pyronin Y (not shown). The bands were absent in the H fraction if it was treated with ribonuclease but were present if placental ribonucleasin was added to the H fraction before the ribonuclease was added. No RNA bands were observed in the B and R fractions (Fig. 3). We think the results in Figs. 2 and 3 provide convincing evidence that RNA is present in the H fraction. The RNA is degraded by ribonuclease, and the degradation can be blocked by ribonucleasin. The protein bands that migrated more slowly were not affected by ribonuclease treatment (Fig. 3).

To find out if the RNA moiety in the H fraction participates in the synthesis of ALA, we added ribonuclease to the crude ALA-synthesizing enzymes or treated the H fraction with ribonuclease for 20 minutes before adding the B fraction. We found that synthesis activity was reduced in both cases by ribonuclease (Table 1). Reduction of the ALA-synthesizing-enzyme activity by ribonuclease can be blocked by the addition of ribonucleasin to the reaction mixture (Table 1). The addition of deoxyribonuclease also caused a slight decrease of enzyme activities. We think this was caused by contaminating ribonuclease in the deoxyribonuclease, because the presence of ribonucleasin blocked the reduction of synthesis activities by deoxyribonuclease (Table 1).

Since (i) the conversion of glutamate to ALA (by the crude enzymes) and to GSA (by H + B) was reduced by the addition of ribonuclease and (ii) the analysis by gel electrophoresis showed that ribonuclease treatment did not affect the major protein bands, we conclude that RNA present in the H fraction is needed for the synthesis of ALA from glutamate. Furthermore, we have preliminary evidence that RNA extracted from the H fraction can replace the H fraction in the conversion of glutamate to GSA. We believe that RNA alone is the needed component in the H fraction for ALA

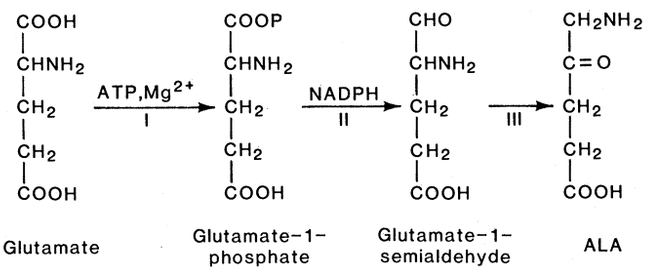

Fig. 1. The postulated pathway for the formation of ALA from glutamate (*4*).

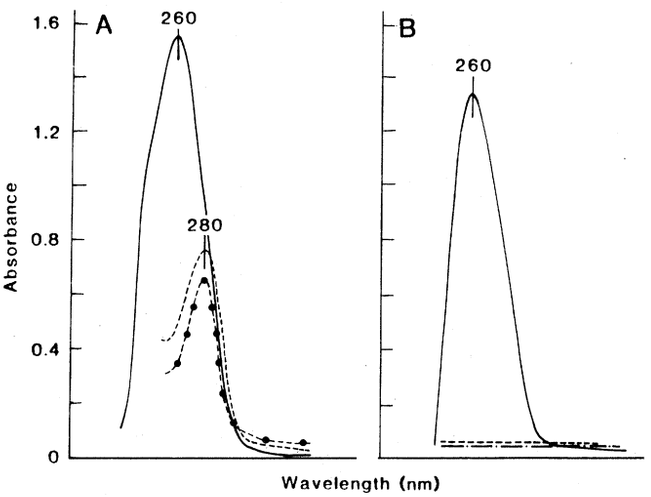

Fig. 2. (A) Absorption spectra of the heme-bound fraction (——), the Blue-bound fraction (- - - -), and the runoff fraction (●). ALA-synthesizing enzymes were isolated from light-grown wild-type cells of *Chlamydomonas* and separated into the three fractions as described by Wang *et al.* (*3*). (B) Absorption of supernatants at 260 nm after the heme-bound fraction has been precipitated by cold $HClO_4$. Absorption at 260 nm remained in the supernatant if the sample was treated with ribonuclease for 20 minutes at 29°C before acid precipitation (——). If heme-bound fraction was incubated with ribonucleasin before treatment with ribonuclease (----) or not treated in any way (⌣), the absorption at 260 nm was absent in the supernatant after acid precipitation.

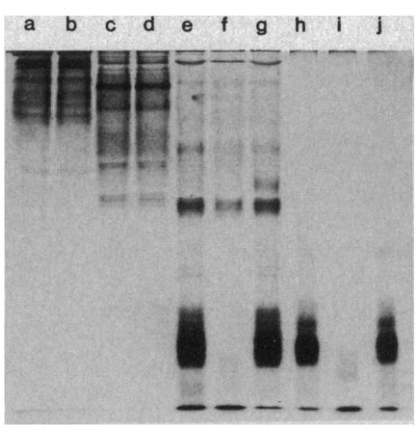

Fig. 3. Silver-stained, polyacrylamide gel (10 percent), without SDS. The gel was run first at 10 mA for 4 hours. The current is 10 mA during stacking and 20 mA during the run. Protein concentrations of H (the heme-bound fraction), B (the Blue-bound fraction), and R (the runoff fraction) were determined by the Lowry method (*12*) and 20 μg of protein from each fraction was placed on the gel. (Lane a) B fraction; (lane b) B fraction + 1 μg of ribonuclease; (lane c) R fraction; (lane d) R fraction + 1 μg of ribonuclease; (lane e) H fraction; (lane f) H fraction + 1 μg of ribonuclease; (lane g) H fraction + 1 μg of ribonuclease + 500 units of ribonucleasin; (lane h) yeast tRNA; (lane i) yeast tRNA + 1 μg of ribonuclease; (lane j) yeast tRNA + 1 μg of ribonuclease + 500 units of ribonucleasin.

synthesis. Because both the crude enzyme preparation and the combined H and B fractions were free of ribosomes, we are certain that the participation of RNA in formation of ALA was ribosome independent. Involvement of RNA in processes other than the ribosome-dependent protein synthesis has been reported in only a few other instances. An RNA moiety is essential for the functions of ribonuclease P of *Escherichia coli* (*7*) and of signal recognition particles in microsomes (*8*). Aminoacyl-tRNA can add aminoacyl moieties to the α-amino group on the terminal amino acid residue of proteins (*9*).

The proposed pathway for ALA synthesis mentioned above requires that the α-carbon of glutamate be activated. We propose that the activation of glutamate is accomplished by a tRNA-like molecule in the H fraction and an aminoacyl synthetase in the B fraction. We further suggest that the activated glutamate (which could be a glutamyl RNA molecule) is converted to GSA by one or more enzymes in the B fraction. The RNA participating in ALA synthesis has also been isolated from barley plastids and was found to hybridize to the barley plastid genome (*10, 11*).

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Functional Properties of Antigen-Specific T Cells Infected by Human T-Cell Leukemia-Lymphoma Virus (HTLV-I)

Abstract. *Tetanus-toxoid specific helper-inducer T-cell clones, which had been infected and transformed by human T-cell leukemia-lymphoma virus (HTLV-I), were obtained from an antigen-specific human T cell line by using a limiting dilution technique in the presence of the virus. These HTLV-I–infected T-cell clones proliferated specifically in response to soluble tetanus toxoid but, unlike normal T cells, they could do so in the absence of accessory cells. The HTLV-I–infected T-cell clones did not present the antigen to autologous antigen-specific T cells that were not infected with HTLV-I. The capacity of helper-inducer T cells to retain antigen-specific reactivity after infection by HTLV-I, while losing the normal T-cell requirement for accessory cells, has clinical and theoretical implications.*

The term human T-cell leukemia-lymphoma virus (HTLV) refers to a unique family of T-cell tropic retroviruses. Viruses belonging to the HTLV family play a vital role in the pathogenesis of certain adult T-cell neoplasms (*1, 2*) and are believed to be the etiologic agents of acquired immune deficiency syndrome (AIDS) (*3, 4*). A well-recognized property of some subtypes of HTLV is the ability to infect and transform T cells from normal umbilical cord blood and bone marrow in vitro (*5, 6*). However, the effects of HTLV on antigen-driven T-cell responses are not well understood.

We established a tetanus-toxoid reactive T-cell line from a normal blood donor by repeated cycles of in vitro stimulation with soluble antigen and irradiated autologous peripheral blood mononuclear cells (PBM) as a source of accessory cells (*7*). The cells were continuously exposed to T-cell growth factor (TCGF or interleukin-2). We then attempted to propagate clones from this immune T-cell line in the presence and absence of HTLV-I. In the absence of virus, the cloning efficiency was approximately one in 64; however, in the presence of the virus, it was one in five, as detected by Poisson analysis of large numbers of replicate microcultures set up with varying numbers of antigen-responsive cells. The most rapidly growing clones were YTA1, which was derived from a well without virus, and YTH3 and YTH5, which were derived from wells containing virus. These clones, which were obtained by limiting dilution under conditions of one cell per well and have been maintained in continuous culture for more than 160 days after cloning, are the subject of the present report.

Clone YTA1 exhibited a substantial proliferative response against soluble antigen but only in the presence of irradiated autologous PBM that served as accessory cells. In contrast, YTH3 and YTH5 replicated spontaneously without TCGF or accessory cells. Moreover, YTH3 and YTH5 substantially increased their rate of proliferation in response to tetanus toxoid in the presence and also the absence of irradiated autologous PBM (Fig. 1). The overall magnitude of response was generally greater in the presence of accessory cells.

The antigen specificity of the proliferation of these clones was studied by using tetanus toxoid, purified protein derivative, streptokinase and streptodornase, and Formalin-inactivated, zonally purified A2/Aichi/68 influenza virus. None of the clones tested responded to antigens other than tetanus toxoid (data not shown). These data taken together suggest that immune T cells cloned in the presence of HTLV-I retained the capacity to recognize and respond to soluble antigen while acquiring the capacity to respond in the absence of accessory cells.

We then infected YTA1 by recloning in the presence of HTLV-I as described earlier. The resultant T cell population, which was propagated under starting conditions of one cell per well by limiting dilution, is referred to as YTA1H. This HTLV-I–infected clone, unlike the original YTA1 clone, replicated spontaneously without TCGF. YTA1H increased its rate of replication upon exposure to soluble antigen in a dose-dependent fashion, and an antigen-driven response was observed in the absence of accessory cells (Table 1).

Clone YTH3 retained the antigen-specific reactivity described above for approximately 120 days in culture following cloning, but the magnitude of the response gradually decreased. Clone YTH5, in contrast, developed within 60 days a high spontaneous rate of replication in the absence of TCGF. The observable antigen-driven proliferative response was lost and could not be restored by recloning (data not shown).

We wished to know whether clones YTH3 and YTH5 were themselves capable of serving as accessory cells in presenting soluble antigen to normal T cells. We observed that irradiated YTH3 and