Proc. Natl. Acad. Sci. USA
Vol. 74, No. 3, pp. 1172–1176, March 1977
Genetics

Proposed involvement of an internal promoter in regulation and synthesis of mitochondrial and cytoplasmic leucyl-tRNA synthetases of *Neurospora**

(promoters/regulation)

PATRICIA M. BEAUCHAMP, EDITH W. HORN, AND S. R. GROSS

Division of Genetics, Department of Biochemistry, Duke University, Durham, North Carolina 27710

Communicated by Norman Giles, December 16, 1976

ABSTRACT **Genetic analysis of an electrophoretic variant of the mitochondrial leucyl-tRNA synthetase [L-leucine:tRNA^{Leu} ligase (AMP-forming), EC 6.1.1.4] indicates that it is either an allele of or linked closely to *leu*-5^{ts}, a mutant that is known to produce a cytoplasmic leucyl-tRNA synthetase with an altered affinity for leucine as well as being deficient in the production of the mitochondrial enzyme. Immunological analysis indicates that the two synthetases have little, if any, structural homology. The pattern of synthesis of the enzymes in *leu*-5^{ts} revertants, the reciprocal relationship of the production of the two enzymes in response to a negative regulatory element, presumably of mitochondrial origin, as well as the lack of detectable structural homology, led to the proposal that the phenotype of *leu*-5^{ts} results from a mutational alteration in the structural gene for the cytoplasmic enzyme in a region involved in the initiation of transcription of the adjacent gene for the mitochondrial enzyme.**

Previous investigations of the temperature-sensitive mutant 45208t (*leu*-5^{ts}) implicated the involvement of the *leu*-5 region of linkage group V of *Neurospora crassa* in the production of both the cytoplasmic and mitochondrial leucyl-tRNA synthetases [L-leucine:tRNA^{Leu} ligase (AMP-forming), EC 6.1.1.4] (1, 2). The mutant, unable to grow at temperatures in excess of 39° and auxotrophic for leucine at intermediate temperatures, was shown to produce a cytoplasmic leucyl-tRNA synthetase with a lower affinity for leucine and to be error prone in the sense that a large fraction of the protein produced at elevated temperatures is defective (1, 3). The most impressive feature of the mutant's phenotype, however, is the virtual absence of the mitochondrial leucyl-tRNA synthetase (2, 4). An analysis of revertants of 45208t (2) confirmed the previous genetic analysis that a single genetic alteration in the *leu*-5 region was responsible for the altered affinity for leucine of the cytoplasmic enzyme and failure to produce the mitochondrial enzyme. In this report evidence is presented which suggests that the *leu*-5 region is comprised of two adjacent genes specifying the structure of the cytoplasmic and mitochondrial enzymes. We propose that alteration of the cytoplasmic enzyme and loss of mitochondrial enzyme production by 45208t results from a change in the structural gene for the cytoplasmic enzyme in a region involved in initiating transcription of the adjacent gene specifying the structure of the mitochondrial enzyme.

MATERIALS AND METHODS

The strains of 45208t used are derivatives of the original temperature-sensitive leucine auxotroph isolated by Beadle and Tatum (5). They have been made reasonably isogenic to the standard strain, STD8A, by two successive backcrosses. Revertants of 45208t-2-15A were obtained as described by Weeks and Gross (2) except that selection was on leucine-supplemented medium (150 μg/ml) at 39°.

Crude preparations of leucyl-tRNA synthetase were obtained as described by Gross *et al.* (4) as mixtures of both the cytoplasmic and mitochondrial enzymes. The filter binding assay as modified by Weeks and Gross (2) was generally used to measure leucyl-tRNA synthetase aminoacylating activity. Specific activities and K_ms were determined by measuring activity at 15 sec intervals for the first 2½ min of the reaction. The K_ms reported here differ somewhat from those published previously. This is probably due to a combination of a change in data processing (all initial velocities were calculated by linear regression analysis) and a change in quality of some of the commercially obtained reagents, especially *Escherichia coli* tRNA.

Antibody to the cytoplasmic leucyl-tRNA synthetase was obtained from New Zealand White rabbits that were injected subcutaneously with an emulsion of 1 mg of purified enzyme and complete Freund's adjuvant. The rabbits were boosted with purified enzyme–adjuvant emulsion 1 month after the first injection and serum with anticytoplasmic-enzyme activity was obtained 1 week later. Antibodies to the mitochondrial enzyme were obtained essentially as above using a mitochondrial enzyme preparation that was purified until free of the cytoplasmic enzyme.

Titration of antibody activity was performed using the globulin fraction precipitating below 40% saturation with ammonium sulfate. Globulin dissolved in 0.05 M KPO_4 at pH 7.7 was dialyzed against 500 volumes of the same buffer overnight. The dialysate was centrifuged at 20,000 × *g* to remove undissolved material. One milliliter of enzyme and 1 ml of antibody at the desired protein concentration were incubated at 4° for 4–5 hr. The mixture was centrifuged at 12,000 × *g* for 30 min and 0.5–0.7 ml was carefully removed from the top and assayed in duplicate for enzyme activity.

RESULTS

The cytoplasmic leucyl-tRNA synthetase has been purified to near homogeneity and consists of a single 110,000–115,000 dalton polypeptide (6). The mitochondrial enzyme has not been completely purified but its chromatographic behavior is sufficiently different from that of the cytoplasmic enzyme so that it could be freed of it by successive chromatography on brushite, DEAE-cellulose, and hydroxylapatite. The sedimentation velocity in glycerol gradients of the mitochondrial enzyme is that of a 90,000–95,000 dalton globular protein (6). The cytoplasmic and mitochondrial enzymes differ not only in size and chromatographic mobility but also in their affinity for leucine, tRNA specificity, salt concentration dependence, and heat stability (2).

* *Dedicated to the memory of Edward L. Tatum.*

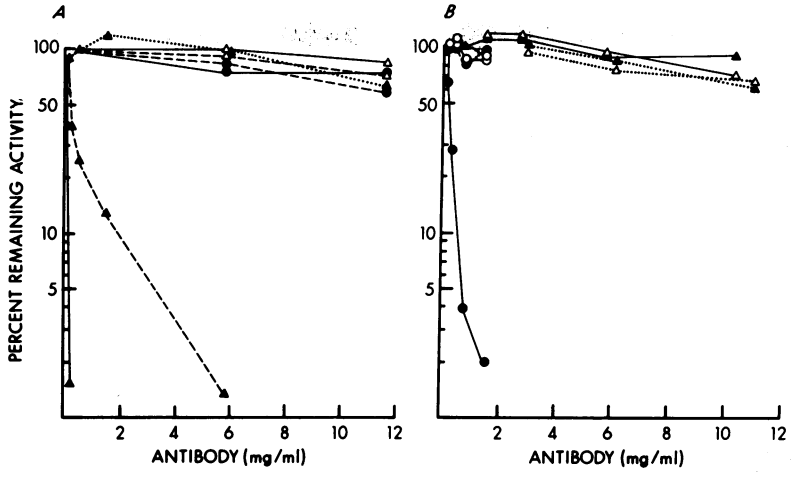

FIG. 1. Antibody inhibition of leucyl-tRNA synthetase. (*A*) Inhibition by globulin antibodies to cytoplasmic leucyl-tRNA synthetase. ▲, Cytoplasmic leucyl-tRNA synthetase; ●, mitochondrial leucyl-tRNA synthetase; △, cytoplasmic phenylalanyl-tRNA synthetase; — and - - - - represent two different independently obtained anticytoplasmic leucyl-tRNA synthetases; and· · · · represents control antibody. (*B*) Inhibition by globulin antibodies to mitochondrial leucyl-tRNA synthetase. ○, Mitochondrial phenylalanyl-tRNA synthetase; all other symbols are as in *A* above.

The three observations requiring interpretation are: (*i*) the *leu*-5^{ts} mutation results in the production of cytoplasmic tRNA synthetase with an altered K_m for leucine with the concomitant loss of the ability to synthesize the mitochondrial enzyme; (*ii*) the cytoplasmic enzyme is about 1.2 times the size of the mitochondrial enzyme; (*iii*) both enzymes are capable of charging mitochondrial tRNA. The most reasonable models that could account for the observations suggest either that the *leu*-5 gene specifies a subunit common to both enzymes, which does not yield active mitochondrial enzyme when genetically altered, or, alternatively, that the mitochondrial enzyme is derived from the cytoplasmic enzyme by some processing reaction that cleaves off about 20% of the amino acid residues from one or both ends. According to the latter notion the change in K_m of the cytoplasmic enzyme as well as the absence of the mitochondrial enzyme might result from a change in cleavage pattern, a change that would have to be subtle because the sedimentation rate and electrophoretic properties of the *leu*-5^{ts} cytoplasmic enzyme are essentially normal (unpublished observation). The fact that the cytoplasmic enzyme is a single polypeptide chain seems to rule out the subunit model, but both models imply that the enzymes must have extensive regions of homology.

One would expect extensive structural homology to be reflected in antigenic crossreactivity. Rabbit anticytoplasmic leucyl-tRNA synthetase was obtained using highly purified enzyme as the antigen. Double diffusion analysis yielded only a single major precipitation band and two barely visible minor bands upon interaction of the antibody with the purified enzyme. Antibody prepared against the mitochondrial leucyl-tRNA synthetase purified free of the cytoplasmic enzyme was heterogeneous, yielding several bands when tested by double diffusion against the mitochondrial enzyme. The two enzymes appeared immunologically unrelated as judged by the immunodiffusion precipitin reaction. No immunoprecipitate was observed when antibody to the cytoplasmic leucyl-tRNA synthetase was tested with the mitochondrial enzyme, nor did overlapping diffusion of the mitochondrial enzyme alter the precipitation pattern of the cytoplasmic enzyme and its antibody. Similarly, the antimitochondrial antibody did not yield precipitation bands when tested against the cytoplasmic enzymes, nor were any of the precipitin lines produced by the mitochondrial enzyme–antimitochondrial-antibody interaction disturbed by overlapping diffusion of the cytoplasmic enzyme.

Results of a more quantitative test of antigenic homology between the cytoplasmic and mitochondrial leucyl-tRNA synthetases are illustrated in Fig. 1. The two independently derived rabbit preparations of antibody to cytoplasmic leucyl-tRNA synthetase interacted strongly with the cytoplasmic enzyme, as judged by the loss of enzymatic activity, without having a significant effect on the activity of either the mitochondrial enzyme or the phenylalanine cytoplasmic enzyme, which served as a control. Similarly, the antimitochondrial leucyl-tRNA synthetase antibody did not crossreact with the cytoplasmic enzyme. A correspondingly high degree of specificity and lack of crossreactivity was noted when the ATP–PP$_i$ exchange reaction was measured. Immunoaffinity chromatography of the cytoplasmic and mitochondrial leucyl-tRNA synthetases on the antibodies coupled to CNBr-activated Sepharose (7) also failed to reveal any antigenic homology between the two enzymes. It should be pointed out that the corresponding enzymes from *Tetrahymena* also show no mutual antigenic crossreactivity (8).

The question of genetic and structural homology was pursued further by searching for a strain of *Neurospora* that produces structurally modified, functionally active tRNA synthetases. The simple notion here is that modification of a sequence common to both enzymes would be detectable in both the cytoplasmic and mitochondrial enzymes. A direct search for temperature-sensitive leucyl-tRNA synthetase mutants by the inositol-less death procedure (9) failed to yield useful mutants. However, an examination of several strains of *N. crassa* isolated from nature yielded one, Mauriceville-1cA (isolated near Mauriceville, Texas, by D. D. Perkins), that produces variable, but generally low amounts of mitochondrial enzyme activity. This enzyme was easily distinguished from that of our standard strain by its greater electrophoretic mobility in polyacrylamide gels (Fig. 2). The cytoplasmic enzyme from the Mauriceville strain is electrophoretically indistinguishable from that of our standard strain.

The existence of an electrophoretic variant of the enzyme provided the means to test directly whether the *leu*-5 region contained the information that specified the structure of the

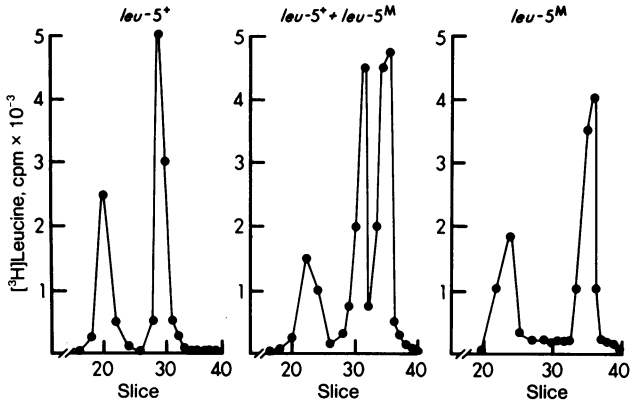

FIG. 2. Electrophoretic mobility of leucyl-tRNA synthetases from strains *leu*-5^{ts}, Mauriceville-1cA (*leu*-5^{M}), and a mixture of the two. The anode is to the right. The mitochondrial enzyme moves toward the anode faster than the cytoplasmic enzyme. The enzyme preparations used were enriched for the mitochondrial enzyme by ammonium sulfate precipitation (55% saturation). Electrophoresis was in a continuous buffer system of 0.037 M glycine adjusted to pH 8.2 with Tris in 9 cm 7.5% polyacrylamide gels. After a 1 hr prerun at 2 mA per tube, 0.2 mg protein in 0.1 ml 20% (vol/vol) glycerol was applied and run for 3 hr at 2 mA per tube. Gel slices (1 mm) were placed in 0.1 ml of 0.05 M Tris·HCl buffer, pH 7.2, containing 0.5 mM dithiothreitol and protein was allowed to leach from the gel overnight at 4°, then assayed.

mitochondrial enzyme. Three crosses were performed to test whether the genetic determinant of the mitochondrial enzyme from Mauriceville (*leu*-5^{M}) is in the *leu*-5 region. The analysis unfortunately was limited because the phenotype of each segregant could only be scored after electrophoresis and assay of its mitochondrial leucyl-tRNA synthetase.

Twenty segregants, 10 *inl*$^{+}$ and 10 *inl*$^{-}$ from a cross, *leu*-5^{M} *inl*$^{+}$ × *leu*-5^{+} *inl*$^{-}$, were analyzed. Only two recombinants were found, one *leu*-5^{M} *inl*$^{-}$ and one *leu*-5^{+} *inl*$^{+}$. This indicates that *leu*-5^{M} is linked to and approximately 10 map units from *inl*, a gene in linkage group V known to be 10–15 map units from *leu*-5 (1). *Leu*-5^{M} *inl*$^{+}$ was then crossed to *leu*-5^{ts} *inl*$^{-}$ and 10 temperature-insensitive *inl*$^{-}$ recombinants were analyzed. All produced a mitochondrial enzyme with the electrophoretic mobility of *leu*-5^{M}. To improve resolution and minimize bias because of the two possible orientations of *leu*-5^{ts} and *leu*-5^{M} with respect to outside markers, 20 double recombinants (*lys*-1^{+} temperature-insensitive *inl*$^{-}$) were analyzed from a cross of *lys*-1^{-} *leu*-5^{M} *inl*$^{+}$ by *lys*-1^{+} *leu*-5^{ts} *inl*$^{-}$; all produced the *leu*-5^{M} mitochondrial enzyme. Interference from sequence inhomology seemed not to be a problem because the exchange frequency of outside markers observed in this cross was within the range expected for the *lys*-1 to *inl* interval, which has been reported to be 20–30 units long (10). Because the limit of resolution of the analysis of double recombinants from this cross is of the order of 2 units, *leu*-5^{M} is close to, if not allelic with, *leu*-5^{ts}.

Much of the genetics of the original *leu*-5^{ts} mutant, 45208t, has been deduced by reversion analysis. We reported previously (2) that prototrophic revertants could be obtained by selection for growth at 39° on minimal medium after irradiation with ultraviolet light. 45208tR5 (R5), a revertant obtained this way, was found to produce both the cytoplasmic and mitochondrial enzymes. Since genetic analysis indicated that the reversion event occurred within the *leu*-5 region and small differences were found between the enzymes from the revertant and our standard strain, it seemed likely that R5 was a secondary intragenic suppressor mutation that restored both enzyme activities. If instead of selecting simultaneously for reversion of temperature sensitivity and leucine auxotrophy, selection is applied only for reversion of temperature sensitivity by plating mutagen treated conidia of 45208t on leucine-containing medium at 39°, a much larger class of revertants is obtained, typified by 45208tR9 (R9) and 45208tR143 (R143). R9, a revertant that grows at about one-half wild-type rate on leucine at 39°, behaves genetically as though a mutational event occurred within or very near the *leu*-5 region (no *leu*-5^{ts} segregants were obtained from among 568 segregants from crosses of R9 by *leu*-5^{+}). Despite the fact that it grows at 39° on leucine, R9 produces no more mitochondrial leucyl-tRNA synthetase activity than the *leu*-5^{ts} from which it was derived. However, the cytoplasmic enzyme is produced in near normal amounts and has a much lower K_m for leucine than *leu*-5^{ts} does (Table 1).

The revertants of the class typified by 45208tR143 grow on leucine at about one-half the wild-type rate at permissive temperatures and at less than one-tenth the wild-type rate at 39° (at restrictive temperatures the requirement for leucine of R143, like that of R9, is absolute). Genetic analysis of R143 indicated the involvement of a suppressor mutation near *bis* (biscuit) 20–30 units distal to *leu*-5 on linkage group V. As indicated in Table 1, the K_m for leucine of the cytoplasmic leucyl-tRNA synthetase produced by the suppressor-bearing 45208tR143 is no different from that of the *leu*-5^{ts} from which it was derived. Suppression by *sup*143 does not increase the level of mitochondrial leucyl-tRNA synthetase production above the trace amount characteristic of the original *leu*-5^{ts} mutant.

The electrophoretic heteromorphism observed between the STD8A and Mauriceville mitochondrial enzymes suggested the possibility that some genetic difference between STD8A, our standard strain derived from *inl* 89601, and the strains used originally in the isolation of the *leu*-5^{ts} mutant 45208t may be the basis for the differences in K_m of the cytoplasmic enzyme and absence of mitochondrial enzyme activity. Strain 45208t

Table 1. K_m values for leucine of the cytoplasmic leucyl-tRNA synthetase in various strains

Strain	Genotype	K_m, μM (± SEM)	Number of determinations
STD8A	*leu*-5^{+}	14.3 (± 2.0)	6
Mauriceville-1cA	*leu*-5^{M}	14.1 (± 0.5)	2
Abbott 4A	*leu*-5^{A}	18.7 (± 0.2)	4
Lindegren 25a	*leu*-5^{L}	20.9 (± 0.9)	4
45208t-2-15A	*leu*-5^{ts}	55.5 (± 11.5)	4
45208tR143-2-59A	*leu*-5^{ts} *sup*143	55.1 (± 11.1)	4
45208tR5A	*leu*-5^{ts} reversion R5	23.0 (± 1.4)	2
45208tR9-2-108A	*leu*-5^{ts} reversion R9	19.9 (± 3.0)	5

was isolated originally from an ascospore obtained from a cross of Lindegren 25a and Abbott 4A. Conidia of one of the parents were irradiated but, from the published account (5), it is not possible to determine which parent contributed linkage group V. Hence, the cytoplasmic and mitochondrial enzymes from the original parents were compared to those from the *leu*-5^{ts} mutant and our standard strain. No electrophoretic difference could be found between the mitochondrial enzymes produced by the three wild-type strains. As indicated in Table 1, however, the K_ms of the cytoplasmic enzymes from both Lindegren 25a and Abbott 4A appear somewhat higher than that of STD8A, but only that of Lindegren 25a is significantly higher (P = <0.05, Student's t Test, unpaired) than the corresponding enzyme from STD8A. The K_ms of the R5 and R9 mutants fall into the Abbott–Lindegren range, which suggests the likelihood that the enzyme from one of these wild-type strains is more appropriate than STD8A as a reference for an analysis of the structural basis of the *leu*-5^{ts} mutation.

Measuring the electrophoretic mobility of the cytoplasmic enzyme seems to be much less sensitive than other methods for detecting heteromorphism. No significant difference in electrophoretic mobility of the native cytoplasmic enzyme has been found between mutant, revertants, and 37 different wild-type strains isolated from widely divergent geographic regions, despite the fact that a great deal of variation in specific activity and stability was observed. Furthermore, the cytoplasmic enzymes from *N. sitophila*, *N. tetrasperma*, and *N. africana* were electrophoretically indistinguishable from the enzyme from STD8A. Evidently constraints on charge, shape, or size of cytoplasmic enzyme are greater than on the mitochondrial enzyme.

DISCUSSION

The sum of the immunological and structural information provided here suggests strongly that the cytoplasmic and mitochondrial leucyl-tRNA synthetases of *Neurospora* do not share extensive regions of homology. Hence it is difficult to maintain the view that the structure of both enzymes, in large measure, is specified by the same gene. However, another line of evidence is probably more instructive in suggesting how the *leu*-5^{ts} mutation might simultaneously result in the production of a cytoplasmic enzyme with an altered affinity for leucine and failure to produce a significant amount of the mitochondrial enzyme.

The synthesis of many mitochondrial proteins specified by nuclear genes is stimulated when mitochondrial protein synthesis is inhibited (11). We have shown elsewhere (12) that the synthesis of the mitochondrial leucyl-tRNA synthetase is increased as much as 5- to 7-fold when mitochondrial protein synthesis is inhibited by chloramphenicol or ethidium bromide. It was observed that concomitant with the increase in the mitochondrial enzyme the specific activity of the cytoplasmic enzyme decreases upon prolonged incubation (about 20 hr, or 4 to 5 generation equivalents) in the presence of chloramphenicol. Although the reciprocal relationship between the two enzymes might be taken to imply the derivation of the mitochondrial enzyme from the cytoplasmic enzyme or some common precursor, we were able to show that the increase in mitochondrial enzyme production was dependent upon cytoplasmic protein synthesis. No such increase in mitochondrial enzyme activity occurred in the presence of cycloheximide with or without chloramphenicol (12). This precludes the participation of some constitutive enzymatic interconversion reaction, though such processing reactions clearly take place in *Neurospora*. We found, for example, that the specific activity of the mitochondrial phenylalanyl-tRNA synthetase does increase when both cytoplasmic and mitochondrial protein synthesis are turned off by combined chloramphenicol and cycloheximide treatment (12). These observations then suggest that if there is any relation between the mitochondrial and cytoplasmic leucyl-tRNA synthetases it must be at the level of either messenger RNA synthesis or some step in the processing of messenger once synthesized.

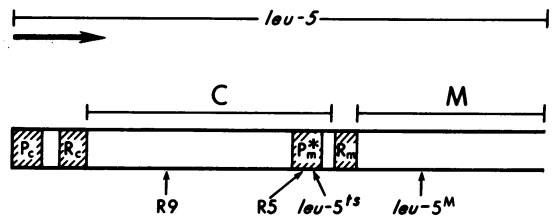

FIG. 3. Proposed model of the *leu*-5 region. See *text* for definition of symbols.

A model to explain the relationships observed between structure and synthesis of the mitochondrial and cytoplasmic leucyl-tRNA synthetases is presented in Fig. 3. The model is based upon the genetic evidence indicating that the gene specifying the Mauriceville electrophoretic variant of the mitochondrial enzyme is either allelic with or very close to the gene specifying the K_m variant of the cytoplasmic enzyme. The essential feature of the model is the postulation of a region, P^*_m, within the structural gene for the cytoplasmic enzyme (C), which is involved in the initiation of transcription of the adjacent gene of the mitochondrial enzyme (M). We refer to it as P^*_m to avoid the unnecessary implication that it is the site of RNA polymerase binding but to imply, instead, that it is at the very least, a region structurally involved in initiation of transcription by RNA polymerase. Jackson and Yanofsky (13) have postulated such an overlap of a structural gene and internal promoter in the tryptophan operon of *E. coli*.

The model also includes a recognition site (R_m) for a negative regulatory element of mitochondrial origin of the sort postulated by Barath and Kuntzel (11). Because this regulatory element controls the rate of synthesis of many mitochondrial proteins specified by nuclear genes, it must be repeated quite frequently in the genome. We postulate also that the direction of transcription (polarity) is from left to right, that is, that P^*_m is near the region specifying the carboxy terminus of the cytoplasmic enzyme because, normally, about 8 to 10 times more cytoplasmic enzyme is produced than mitochondrial enzyme. Although we have no specific evidence for the existence of P_c and R_c, the promoter and regulatory site for the gene specifying the cytoplasmic enzyme (C), they are included for the sake of symmetry.

The model neatly accounts for the enzymological pleiotropy of *leu*-5^{ts} by implying that the mutational event occurs in the P^*_m region of C, which upon translation results in the production of a cytoplasmic enzyme with a lower affinity for leucine while at the same time rendering P^*_m ineffective in promoting transcription of the mitochondrial enzyme gene M. The model also can account for the two kinds of revertants of 45208t observed. Accordingly, the revertant R5, which simultaneously regained the ability to produce the mitochondrial enzyme and a cytoplasmic enzyme with near normal affinity for leucine (2), probably represents a mutation at or near the original mutation in the P^*_m region. On the other hand, R9, which produces a cytoplasmic enzyme with near normal affinity for leucine but no detectable mitochondrial enzyme, is likely to involve a second-site mutation outside the P^*_m region, which results in a

compensatory alteration in the leucine binding site of the cytoplasmic enzyme. A second-site mutation would not be expected to have a structural effect on the mutationally altered P^*_m region, while it might affect binding properties of the completed protein. The suppressor mutation *sup*143, which is distant from the *leu*-5 region, corrects neither the loss of promoter function at P^*_m nor the affinity for leucine of the cytoplasmic enzyme. It probably involves an alteration in the translation mechanism that partly reverses the temperature-dependent mistranslation that is characteristic of the original *leu*-5^{ts} mutation. The involvement of some general nondispensible function in *sup*143 is suggested further by the fact that strains bearing *sup*143, even when freed of *leu*-5^{ts}, grow slowly irrespective of supplement.

The model presented for the organization of the *leu*-5 region has at least one novel feature with regard to the regulation of the production of the two enzymes. The existence of P^*_m is postulated in order to accommodate the pleiotropic phenotype of the *leu*-5^{ts} mutant 45208t, that is, the failure of the mutant to produce more than a trace of the mitochondrial enzyme and the alteration in the K_m of the cytoplasmic enzyme. Occasional transcription through the P^*_m and R_m sites, yielding normal mitochondrial enzyme, might be expected to occur and perhaps this, rather than contamination by revertants during growth, accounts for the trace of mitochondrial enzyme usually found in extracts of *leu*-5^{ts} (4). However, it seems clear that if, as assumed throughout this discussion, the basis for the phenomena described is related to transcription rather than translation, under normal circumstances, two distinct messages must be produced, one initiated at P_c and the other at or near P^*_m. The interesting consequence of this is that binding of RNA polymerase at P^*_m would interfere with completion of transcription of the cytoplasmic message by RNA polymerase that had initiated transcription at P_c. Similarly, active transcription of the P^*_m by RNA polymerase that had initiated transcription at P_c would interfere with initiation of transcription of the *M* gene.

As stated above, inhibition of synthesis of the putative negative regulatory element of mitochondrial origin by prolonged growth in the presence of chloramphenicol has two effects; it increases the yield of mitochondrial enzyme and decreases the yield of the cytoplasmic enzyme (12). While this reciprocal relationship might be taken as support for our model, there was some concern about the use of antibiotics in establishing the validity of the phenomenon and its quantification. We found recently that the mutant strain *cni*-3, isolated by Rosenberg *et al.* (14), which is partially constitutive for the cyanide insensitive respiratory system by virtue of a maternally inherited mutation (presumably mitochondrial), is also derepressed for the synthesis of the mitochondrial leucyl-tRNA synthetase (Gross and Horn, unpublished). In fact, about 40–50% of the total leucyl-tRNA synthetase activity produced during logarithmic growth is that of the mitochondrial enzyme. This relative increase in activity is accomplished by an approximately 2-fold increase in specific activity of the mitochondrial enzyme and about a 3-fold decrease in specific activity of the cytoplasmic enzyme. While the quantitative relationship between the production of the two enzymes by *cni*-3 may not result wholly from competition between completion of the *C* message and binding of RNA polymerase for initiation of the *M* message, the reciprocal relationship of the synthesis of the two enzymes is in agreement with the prediction by the model of the synthesis of two different messenger RNAs.

It should be clear that the model presented is not the only one that could be constructed to account for the phenomenology of the *leu*-5 region. Transcription of the two cistrons, for example, might be from opposite strands with overlapping central promoter and coding sequences. With special assumptions, the least likely of which is that two leucyl-tRNA synthetases would be obtained, even the recent exciting discovery of Barrell *et al.* (15) that the *D* and *E* genes of bacteriophage ϕX174 are translated from the same RNA sequence in different reading frames can be invoked to explain the lack of structural homology of the two enzymes as well as the phenotype of *leu*-5^{ts} and its revertants. The model of Fig. 3, however, is the preferred one at present, because it appears to accommodate the facts with the fewest assumptions while being amenable to direct test. But, it should be emphasized that all of the models are based upon tight linkage of the genetic determinants of the mitochondrial and cytoplasmic leucyl-tRNA synthetases which, of itself, begs the question whether, in this case, linkage results from a requirement for interdependent, reciprocal regulation of the production of the two enzymes in response to changes in the demands of the cytoplasmic and mitochondrial protein synthetic machinery.

We thank Dr. Harvey Sage and Dr. Meir Fischer for help with the immunology. This work was supported by grants from the National Science Foundation (PCM 74-03018 A02). P.M.B. held an National Institutes of Health predoctoral traineeship during the course of this work.

1. Printz, D. B. & Gross, S. R. (1967) *Genetics* **55**, 451–467.
2. Weeks, C. O. & Gross, S. R. (1971) *Biochem. Genet.* **5**, 505–516.
3. Lewis, C. M. & Holliday, R. (1970) *Nature* **228**, 877–880.
4. Gross, S. R., McCoy, M. T. & Gilmore, E. B. (1968) *Proc. Natl. Acad. Sci. USA* **61**, 253–260.
5. Beadle, G. W. & Tatum, E. L. (1945) *Am. J. Bot.* **32**, 678–686.
6. Beauchamp, P. M. (1975) Ph.D. Dissertation, Duke University.
7. Cuatrecasas, P. (1969) *Biochem. Biophys. Res. Commun.* **35**, 531–537.
8. Chiu, A. O. S. & Suyama, Y. (1973) *Biochim. Biophys. Acta* **299**, 557–563.
9. Lester, H. & Gross, S. R. (1969) *Science* **129**, 572.
10. Strickland, W. N., Perkins, D. D. & Veatch, C. (1959) *Genetics* **44**, 1221–1226.
11. Barath, Z. & Kuntzel, H. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 1371–1374.
12. Beauchamp, P. M. & Gross, S. R. (1976) *Nature* **261**, 338–340.
13. Jackson, E. N. & Yanofsky, C. (1972) *J. Mol. Biol.* **69**, 307–313.
14. Rosenberg, E., Mora, C. & Edwards, D. L. (1976) *Genetics* **83**, 11–24.
15. Barrell, B. G., Air, G. M. & Hutchinson, C. A., III, (1976) *Nature* **264**, 34–41.