

# A mutation resulting in increased triosephosphate isomerase activity in *Mus musculus*

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(Received 27 April 1990 and in revised form 4 July 1990)

## Summary

A mutation resulting in increased triosephosphate isomerase (TPI) activity in blood was recovered in offspring of procarbazine hydrochloride-treated male mice. Breeding experiments indicated a codominant mode of expression. Compared to the wild type, heterozygous and homozygous mutants have mean erythrocyte TPI activities of approximately 140 and 190%, respectively. Besides blood and erythrocytes the increased activity is expressed to a similar degree in spleen, and to a lesser degree in liver, lung, kidney, muscle and brain. Enhanced activity was absent in the heart. Heterozygous and homozygous mutants are viable, fully fertile and exhibit no significant differences in haematological or other physiological traits studied. Biochemical investigations of TPI in both mutant genotypes revealed neither physicochemical nor kinetic differences compared to the wild type. Moreover, immunoinactivation studies showed no difference in the amount of antiplasma required to inactivate a constant amount of TPI activity in all three genotypes, strongly suggesting that the differences in enzyme activity are attributable to differing amounts of enzyme protein expressed per cell. Mapping studies indicated that the mutation is closely linked to the *Gapd* locus and consequently is located either adjacent to or within the *Tpi-1* structural locus. It is hypothesized that the mutation affected a regulatory element contiguous to the *Tpi-1* structural locus which acts by increasing the amount of TPI expressed.

## 1. Introduction

Among the numerous cases of genetically transmitted alterations in enzyme function described in humans only a few examples of increased enzymatic activity are known. Cases with hyperactivity of glucose-6-phosphate dehydrogenase (Dern, 1966), adenosine deaminase (Valentine *et al.* 1977), phosphoribosyl-pyrophosphate synthetase (Becker *et al.* 1973) and pyruvate kinase (Rosa *et al.* 1981) have been observed. This feature is consistent with the hypothesis that mutations affecting primary sequence and structure of an enzyme most likely lead to a deterioration rather than to an improvement of enzymatic function, especially if the enzyme has been selected for maximized catalytic efficiency during evolution. Triosephosphate isomerase (TPI, D-glyceraldehyde-3-phosphate ketol-isomerase, EC 5.3.1.1) represents such a highly evolved enzyme. This enzyme, which is involved in the glycolytic and gluconeogenic pathway

as well as in glyceride synthesis, is detectable in all tissues in a wide variety of species (Scopes, 1968; Gracy, 1974; Snapka *et al.* 1974). Determination of the free-energy profile from the triosephosphate isomerase catalyzed reaction has shown that the enzyme reached the end of its evolutionary development towards maximal catalytic efficiency, since the maximum flux of substrate is determined by diffusion limitation (Knowles & Albery, 1977).

Several alleles associated with TPI deficiency are known in the human population (see Rosa *et al.* 1985). In contrast, no case with a definitely proven hereditary increase of TPI activity has been described so far in the literature. In the mouse only mutations generating a reduction of TPI activity have been recovered in mutagenicity experiments with ethylnitrosourea (Charles & Pretsch, 1987). However, during screening of offspring of procarbazine hydrochloride- (procarbazine) treated male mice (Ehling *et al.* 1985) a mutant with TPI hyperactivity in blood was discovered. An increase of enzyme activity in this tissue may have several causes, such as improved catalytic function, elevation of the cellular concentration of the enzyme, or reduction of mean cell age of

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the erythrocyte population. Especially in the case of the 'perfect catalyst', TPI, an improved catalytic efficiency due to a structural gene mutation would be of interest for enzymologists. Furthermore, the elevation of the cellular concentration of TPI molecules as a cause of the increased TPI activity would be significant for work on the regulation of gene expression in higher organisms. Therefore an initial genetic, biochemical and physiological study of the TPI mouse mutant was conducted. The results of this investigation suggested that the mutation acts by increasing the rate of production of a structurally normal enzyme.

## 2. Materials and methods

### (i) Mice

The original male mutant with approximately 140% of wild-type TPI activity in blood was detected in mutagenicity experiments with procarbazine (Ehling *et al.* 1985). Based on the results from the present study the mutant allele was designated *Tpi-1<sup>r</sup>*.

### (ii) Genetical analysis and mapping

Heterozygous mutant offspring were selected and backcrossed at least ten generations to the C3H/El wild-type strain in order to transfer the mutant gene to a defined inbred genetic background. Heterozygous mutants originating from such backcrosses were mated *inter se* (intercrosses) to recover homozygous mutants which were expected to express a greater alteration in

enzyme activity than the heterozygotes. Homozygosity was ascertained genetically in a subset by outcrossing to homozygous C3H/El. In addition, the offspring of these outcrosses served to characterize a pure heterozygous population. A homozygous line was established by mating homozygous mutants *inter se*.

For linkage studies, heterozygous TPI animals were mated with heterozygous mouse mutants having approximately 50% wild-type glyceraldehyde-3-phosphate dehydrogenase (GAPDH) activity (Charles & Pretsch, 1987), and which were shown to be mutants of the *Gapd* structural locus (Pretsch, Neuhäuser-Klaus & Merkle, 1990). Double heterozygotes were selected in the offspring and backcrossed with C3H/El wild-type inbred mice. Offspring of these crosses were classified on the basis of GAPDH and TPI activity, respectively. Animals which could not be unequivocally classified by this method were genetically tested by backcrossing with C3H/El mice.

### (iii) Physiological, immunological and biochemical characterization

Physiological, immunological and biochemical characterization of the mutant essentially followed the procedure previously described in detail (Merkle & Pretsch, 1989). Only definitely classifiable animals were used.

### (iv) Statistics

To classify the animals obtained in breeding experiments, which could not be genetically tested, a

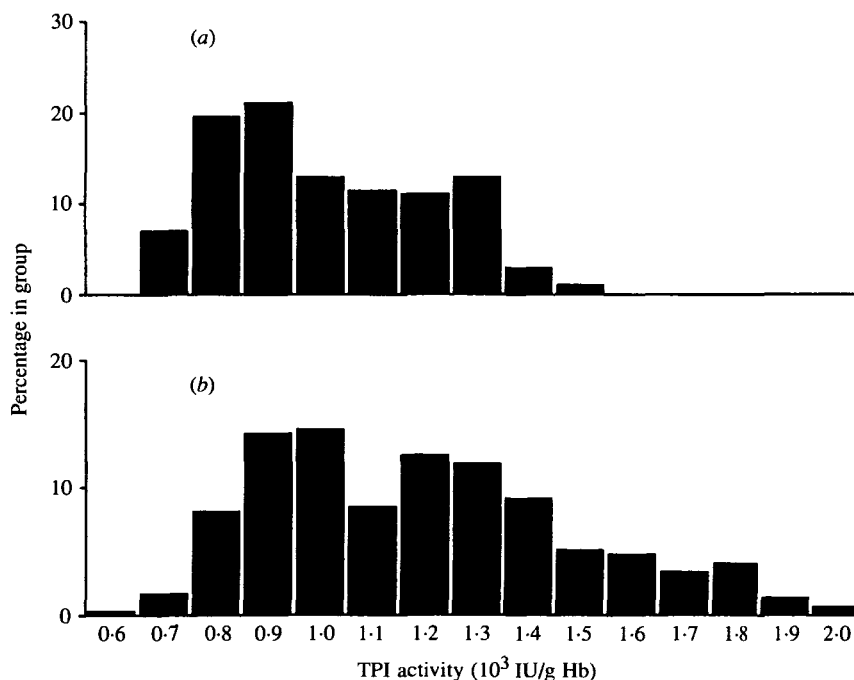


Fig. 1. Distribution pattern of TPI activity in blood of mice obtained in backcrosses between homozygous

C3H/El wild types and heterozygous mutants (a)  $n = 271$ , and in intercrosses between heterozygotes (b)  $n = 296$ .

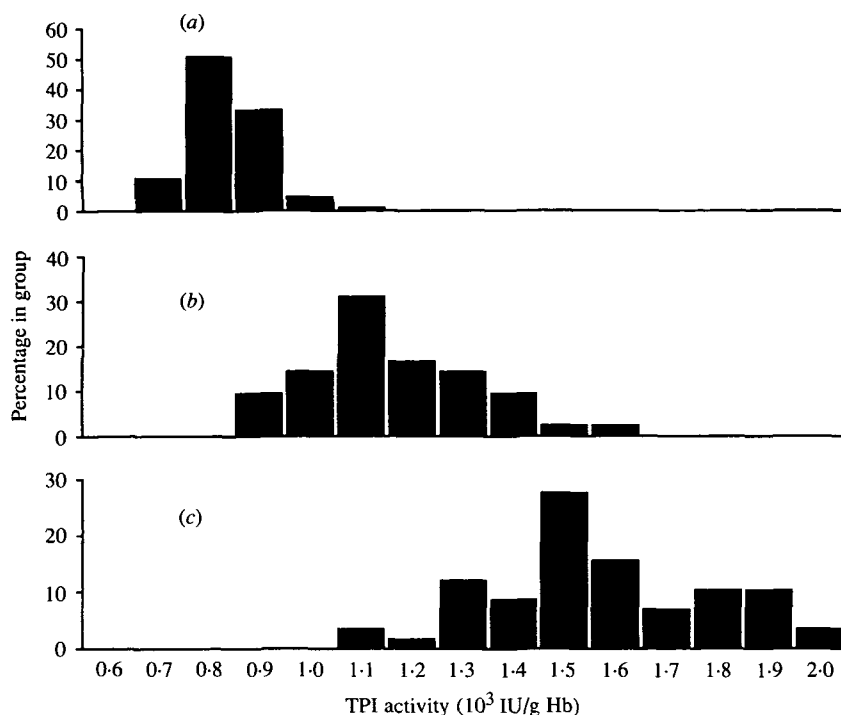


Fig. 2. Distribution pattern of TPI activity in blood observed in animals of pure populations of homozygous

wild types (a)  $n = 114$ , heterozygous (b)  $n = 42$ , and homozygous mutants (c)  $n = 58$ .

discrimination function mainly based on standardized distances was applied as follows:

(observed value – mean value)/standard deviation, related to reference populations of wild type, heterozygous and homozygous mutants. Also the number of different genotypes possible from back- and intercrosses were taken into account; i.e.  $Tpi-1^a/Tpi-1^a$  and  $Tpi-1^a/Tpi-1^r$  from backcrosses and  $Tpi-1^a/Tpi-1^a$ ,  $Tpi-1^a/Tpi-1^r$  and  $Tpi-1^r/Tpi-1^r$  from intercrosses. The classification criterion for offspring of back- or intercrosses was evaluated for the blood TPI activity of a standard population of wild type, heterozygous and homozygous mutants and generated the following classification rule: individuals from back- or intercrosses with a TPI activity less than or equal to 954.4 IU/g haemoglobin (Hb) were classified as homozygous wild types; those with a TPI activity greater than 954.4 IU/g Hb and less than or equal to 1362.1 IU/g Hb were classified as heterozygotes; and, those

with TPI activity greater than 1362.1 IU/g Hb were classified as homozygous mutants. In the biochemical and physiological characterization experiments, data from the same number of males and females were used for the mean and S.E.M. Statistical comparisons between the various genotypes were performed using Student's *t*-test. Differences are considered significant for *P* values less than 0.05. All computations were calculated using SAS<sup>R</sup> programs.

### 3. Results

#### (i) Genetical characterization

Fig. 1 presents the blood TPI activities of litters obtained in backcrosses as well as in intercrosses. No distinct distributions attributable to the different genotypes possible from back- and intercrosses were observed. Based on the classifying rule from the distribution patterns of populations of homozygous wild type, heterozygous and homozygous mutants, respectively (Fig. 2), segregation ratios were calculated for both backcrosses as well as intercrosses (Table 1). The results obtained are consistent with Mendelian expectations. Moreover, the mean litter size observed for the various matings indicated no differences in viability and fertility among the genotypes. However, an increase of variability could be observed in the heterozygous and especially in the homozygous population. This was clearly demonstrated by the increase of the coefficient of variation for TPI activity (standard deviation expressed as percentage of mean) which is 7.7% in the wild-type population, 14.0% in

Table 1. Genetic characterization of a mouse mutant with TPI hyperactivity

Cross	Mean litter size ± S.D.	<i>Tpi-1</i> genotype <sup>a</sup> (n)		
		<i>a/a</i>	<i>a/r</i>	<i>r/r</i>
<i>a/a</i> × <i>a/a</i>	6.2 ± 1.5	186		
<i>a/r</i> × <i>a/a</i>	6.3 ± 1.7	133	138	
<i>a/r</i> × <i>a/r</i>	6.3 ± 2.0	75	143	78
<i>r/r</i> × <i>a/a</i>	6.0 ± 1.6		42	
<i>r/r</i> × <i>r/r</i>	5.6 ± 1.9			58

<sup>a</sup> *a* = *Tpi-1*<sup>a</sup>, wild-type allele; *r* = *Tpi-1*<sup>r</sup>, mutant allele.

Table 2. Physicochemical properties of erythrocyte TPI of homozygous wild type, heterozygous and homozygous mutants with TPI hyperactivity in mice

<i>Tpi-1</i> genotype <sup>a</sup>	Specific activity <sup>b</sup> (% of the wild type)	$K_m$ (GAP) <sup>c</sup> (mM)	Heat stability <sup>d</sup>		pH optimum <sup>c</sup>
			47 °C	50 °C	
<i>a/a</i>	100 ± 3	0.70 ± 0.10	75.0 ± 1.7	46.2 ± 1.5	8.0
<i>a/r</i>	141 ± 4	0.68 ± 0.09	74.5 ± 1.5	46.9 ± 2.0	8.0
<i>r/r</i>	188 ± 6	0.70 ± 0.08	74.5 ± 2.0	46.6 ± 1.3	8.0

<sup>a</sup> Same symbols are used as in Table 1.

<sup>b</sup> Data are given as mean ± S.E.M. of 10 animals.

<sup>c</sup> Data are given as mean ± S.E.M. of 4 animals (double determination).

<sup>d</sup> % remaining activity after 20 min incubation. Data are given as mean ± S.E.M. of 10 animals.

Table 3. Specific activity of erythrocyte TPI and the titre determined by immunoinactivation of homozygous wild type, heterozygous and homozygous mutants with TPI hyperactivity in mice

<i>Tpi-1</i> genotype <sup>a</sup>	Specific TPI activity <sup>b</sup>		Titre <sup>c</sup>	
	IU/g Hb	% of the wild type	IU/ml antiplasma	% of the wild type
<i>a/a</i>	683 ± 21	100 ± 3	231.6 ± 8.4	100 ± 4
<i>a/r</i>	964 ± 27	141 ± 4	230.0 ± 12.2	99 ± 5
<i>r/r</i>	1285 ± 41	188 ± 6	237.1 ± 15.3	102 ± 7

<sup>a</sup> Same symbols are used in Table 1.

<sup>b</sup> Data are given as mean ± S.E.M. of 10 animals.

<sup>c</sup> Data are given as mean ± S.E.M. of 4 animals.

the heterozygous and 16.6% in the homozygous mutant population.

#### (ii) Linkage studies

To study the segregation of *Tpi-1*<sup>r</sup> and *Gapd*, double heterozygotes were backcrossed to C3H/El animals with normal TPI and GAPDH activities. No recombinant in 176 offspring from these crosses were observed implying that these two loci are between 0 and 1.9 cM of each other at the 95% confidence level. Since the *Gapd* locus has been shown to be closely linked to the *Tpi-1* structural locus (Pretsch *et al.* 1990), this result means that the mutant maps near or at *Tpi-1*.

#### (iii) Physicochemical properties

Some physicochemical properties of TPI of heterozygous and homozygous mutants were determined and compared to those of the wild type (Table 2). The specific erythrocyte activity of TPI in heterozygous and homozygous mutants was about 141 and 188% of that of wild-type animals, respectively. TPI from all three genotypes was indistinguishable by various physical, chemical and enzymatic criteria including isoelectric focusing pattern,  $K_m$  for glyceraldehyde-3-

phosphate, heat stability and pH dependence suggesting that the primary structure of the enzyme is identical in both mutant and wild-type forms.

#### (iv) Content of TPI protein

Immunoinactivation experiments with antiplasma specific for TPI were performed to determine if a difference in content of enzyme protein is responsible for the difference in assayable erythrocyte TPI activity of the mutants. Table 3 shows that the titre of antiplasma was not different in both heterozygous and homozygous mutants compared with wild types; that is, a constant amount of antiplasma was required to inactivate the same amount of added enzyme activity. Further, 71 and 53% of the amount of blood of heterozygotes and mutant homozygotes, respectively, were required to obtain an amount of enzyme activity equivalent to that of homozygous wild types. It could be concluded that the amount of TPI in erythrocyte lysate as measured by the enzyme assay in all three genotypes is associated with a comparable difference in the amount of immunologically reactive protein. The data thus are consistent with the hypothesis that the mutation acts by controlling the amount of TPI protein per cell.

Table 4. Specific TPI activity in several tissues of homozygous wild type, heterozygous and homozygous mutants with TPI hyperactivity in mice<sup>a</sup>

<i>Tpi-1</i> genotype <sup>b</sup>	Blood	Liver	Lung	Kidney	Spleen	Heart	Brain	Muscle
<i>a/a</i>	100 ± 3 (787)	100 ± 4 (9633)	100 ± 4 (3003)	100 ± 1 (17771)	100 ± 5 (3794)	100 ± 4 (17614)	100 ± 4 (51768)	100 ± 6 (21789)
<i>a/r</i>	138 ± 3*	124 ± 4*	125 ± 6*	112 ± 3*	146 ± 5*	106 ± 3	113 ± 2*	101 ± 3
<i>r/r</i>	182 ± 6*	149 ± 6*	168 ± 8*	134 ± 6*	188 ± 9*	102 ± 4	127 ± 5*	113 ± 4*

<sup>a</sup>Data are expressed as percentage of TPI wild-type activity and given as mean ± s.e.m. of 12 animals. Significant differences between wild types and mutants ( $p \leq 0.05$ ) are marked by \*. The mean specific TPI wild-type activity is indicated in parentheses as IU/g Hb in the blood, and as IU/g protein in other tissues.

<sup>b</sup>Same symbols are used in Table 1.

Table 5. Haematological data of homozygous wild type, heterozygous and homozygous mutants with TPI hyperactivity in mice<sup>a</sup>

<i>Tpi-1</i> genotype <sup>b</sup>	Haematocrit (%)	Haemoglobin (g/l)	RBC ( $\times 10^{12}/l$ )	MCF (% NaCl)	Glucose-consumption of blood (mg/g Hb/h)
<i>a/a</i>	47.3 ± 0.4	157 ± 2	8.4 ± 0.3	0.496 ± 0.004	2.36 ± 0.27
<i>a/r</i>	47.5 ± 0.8	160 ± 5	8.5 ± 0.2	0.494 ± 0.005	2.25 ± 0.25
<i>r/r</i>	47.2 ± 0.6	155 ± 5	8.4 ± 0.2	0.499 ± 0.004	2.55 ± 0.21

<sup>a</sup>Data are given as mean ± s.e.m. of 8 animals. Abbreviations used: RBC, red blood cells; MCF, mean cellular fragility (NaCl concentration at which 50% of RBCs haemolyse); Hb, haemoglobin.

<sup>b</sup>Same symbols are used in Table 1.

#### (v) Tissue distribution

The activity of TPI has been determined in several tissues both from wild types as well as from heterozygous and homozygous mutants (Table 4). Heterozygous and homozygous mutants have blood TPI activity of approximately 140 and 180% of the wild-type value, respectively. TPI hyperactivity is also expressed in other tissues of both mutant genotypes, however, at varying degrees. In spleen the increase of enzyme activity is approximately the same as in blood while the increase in activity is lower in lung, liver, kidney, muscle and brain. TPI activity of heterozygotes in general is intermediate between that of homozygous wild types and mutants. In heart the increase was found neither in heterozygotes nor in mutant homozygotes. Extractable proteins given in g/100 g wet weight in liver [wild-type value (WTV) = 17.5 ± 1.0], lung (WTV = 13.1 ± 0.4), kidney (WTV = 14.4 ± 0.6), spleen (WTV = 14.4 ± 0.4), heart (WTV = 11.5 ± 0.5), muscle (WTV = 8.3 ± 0.6), and brain (WTV = 8.0 ± 0.4) are not altered in mutants of both genotypes as compared to the wild type.

#### (vi) Erythrocyte enzymes

Beside TPI, nine other enzymes were routinely tested in blood lysate including lactate dehydrogenase (LDH, EC 1.1.1.27), malate dehydrogenase (MDH; EC

1.1.1.37), glucose-6-phosphate isomerase (GPI, EC 5.3.1.9), 3-phosphoglycerate kinase (PGK, EC 2.7.2.3), phosphoglyceromutase (PGAM, EC 2.7.5.3), glyceraldehyde-3-phosphate dehydrogenase (GAPDH, EC 1.2.1.12), glucose-6-phosphate dehydrogenase (G6PD, EC 1.1.1.49), pyruvate kinase (PK, EC 2.7.1.40) and glutathione reductase (GR, EC 1.6.4.2). None of these enzymes exhibit statistically significant differences between the various genotypes.

#### (vii) Physiological data

In accordance to the results from the measurements of the activity of several enzymes other than TPI in blood, the results obtained in the routine haematological tests indicate that the different TPI activity in mutants is not an indirect consequence of an altered red-cell population (Table 5). Other physiological traits such as plasma glucose (WTV = 1.33 ± 0.10 g/l), body weight (WTV = 22.7 ± 0.6 g) and somatic indices of liver (WTV = 5.65 ± 0.13 g/100 g body weight), lung (WTV = 0.55 ± 0.02 g/100 g body weight), kidney (WTV = 1.39 ± 0.05 g/100 g body weight), spleen (WTV = 0.44 ± 0.04 g/100 g body weight) and heart (WTV = 0.40 ± 0.01 g/100 g body weight) also exhibit no differences between wild types and mutants.

#### 4. Discussion

The present study reports the genetical, biochemical and physiological characterization of a chemically induced mouse mutant associated with increased TPI activity in blood. A clear gene dosage effect could be observed. Investigations of haematological and other physiological traits, as well as the study of the activity of other enzymes in blood, demonstrated that the increased TPI activity is not a result of an altered red-cell population or of reduced mean cellular haemoglobin. Moreover, the results revealed no indications for pleiotropic effects, so that the increase of TPI activity may be regarded rather as a specific effect of the mutation.

The specific alteration of enzyme activity as a result of a mutation theoretically may have several causes: (1) structural abnormality producing an enzyme with altered catalytic efficiency, (2) altered rate of production or of degradation of a structurally altered enzyme with normal specific activity, (3) altered rate of production of a structurally normal enzyme due to a regulatory mutation and (4) a combination of these effects. For all of these possibilities examples have been found in mammals (Paigen, 1971). Concerning the mutant described in this paper there is no unequivocal indication for the mechanism which lead to the TPI hyperactivity in blood and several tissues. However, on the basis of the observations that kinetical, physicochemical and immunological properties were indistinguishable between wild types and mutants of both genotypes, a mutation resulting in a structurally altered molecule with improved catalytic characteristics can be excluded. In contrast, the results clearly indicate an increase in the number of TPI molecules in the cell which must be a result of either an increased production or of a decreased degradation rate of the enzyme. From these two possibilities, however, the latter seems rather unlikely, since the unaltered heat stability characteristics of the mutant enzyme may be considered an indication for a normal *in vivo* stability.

Whether the assumed enhanced TPI synthesis is due to a mutational event affecting the structural gene for TPI, i.e. increasing the half life of m-RNA or affecting a regulatory element, remains to be elucidated. Since regulatory gene sites are usually found in close proximity to the structural genes they modulate (Paigen *et al.* 1975), the localization of the mutation at or close to the *Tpi-1* structural gene is consistent with both possibilities. However, the variable and tissue-specific increase in TPI activity which is thought to be encoded by only one gene per haploid genome (Brown *et al.* 1985), the normal physicochemical properties of the mutant enzyme, and the codominant mode of expression implicate a regulatory mutation affecting a *cis*-acting element which exerts its action in a tissue-specific manner. Such positive or negative regulatory elements have been shown to belong to each gene in

an animal cell (Mitchell & Tjian, 1989). The increase of the coefficient of variation of TPI activity in both mutant genotypes might also be interpreted in this manner. A comparable effect was not observed so far in enzyme-activity mutants attributed to mutations of the structural gene of either TPI (Merkle & Pretsch, 1989) or G6PD (Pretsch, *et al.*, 1988). Nevertheless, the definite clarification regarding the site of the mutation and the definite mechanism in which it acts requires further studies. Pulse-labelling experiments using radioactive amino acids, analysis of RNA transcripts and analysis of nucleotide sequence of the affected gene might elucidate the problem. Furthermore, these studies might elucidate the definite nature of the mutation which, based on the mutation induction by procarbazine, is probably a base-pair change (Ehling & Neuhäuser, 1979). The clarification of these problems is of great interest since the knowledge of the relation between mutation and altered enzyme activity in every regulatory mutant may lead to a better understanding of gene expression in higher organisms.

We would like to express our appreciation to U. H. Ehling and J. Favor for discussions and helpful criticism of the manuscript and S. W. Eber for supplying the antiplasma. The competent technical assistance provided by M. Ellen-dorff and S. Wolf is gratefully acknowledged. This study was supported in part by No. B16-E-156-D of the Commission of the European Communities.

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