A GENETIC ANALYSIS OF THE PTERIDINE BIOSYNTHETIC ENZYME, GUANOSINE TRIPHOSPHATE CYCLOHYDROLASE, IN *DROSOPHILA MELANOGASTER*

WILLIAM J. MACKAY AND JANIS M. O'DONNELL

Department of Biological Sciences, Carnegie-Mellon University, Pittsburgh, Pennsylvania 15213

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ABSTRACT

Strains with mutant eye color were surveyed for levels of GTP cyclohydrolase (GTP CH), the first enzyme acting in the biosynthesis of pteridines, the pigments causing red eye color in Drosophila. Six strains were found to have reduced GTP CH activity. In five of the six strains, the reduction of activity is apparent only in the adult head of homozygous mutants. We show that mutations in Punch (2-97, *Pu*) have severe effects on GTP CH activity. In most cases, the reduction of activity is apparent in all tissues and stages that express the enzyme. The activity of GTP CH is shown to be closely correlated with the number of Pu^+ genes in the genome. One ethyl methanesulfonate (EMS)-induced *Pu* mutant has a GTP CH enzyme that is unstable when compared with the wild-type enzyme. Mutations in *Pu* fall into three general classes. The largest class has a recessive lethal and eye color phenotype, 50% or higher GTP CH activity in heterozygotes, and equivalent defects in all tissues. A second class is dominant in eye color phenotype and recessive lethal, with less than 50% GTP CH activity in heterozygotes. The third class is homozygous viable and has severe reduction of activity in the adult head, but no or less severe loss in other tissues.

THE metabolic pathway that is responsible for the biosynthesis of pteridines in *Drosophila melanogaster* is at least bifunctional. Regulation of the pathway presents an interesting developmental problem because its functions place quite different demands on the system, in terms of the quantities and types of pteridines required and in terms of temporal and spatial specificity. Although some pteridines appear to be present throughout the life cycle of Drosophila, there are two periods of larger accumulations, the first, beginning in late larval life, and the second, beginning 70–72 hr after the onset of pupation (FAN *et al.* 1976; PHILLIPS and FORREST 1980). The latter increase in pteridines occurs as they are deposited in pigment granules in the secondary pigment cells of the ommatidia. The concentration of pteridines in these cells is far greater than in any other tissue at any time in the life cycle. Pteridines share the responsibility for the characteristic reddish color Drosophila eyes with another class of eye pigments, the ommochromes. The molecules serve as screening pigments, absorbing laterally deflected light and thus contributing to the visual

acuity of the organism (ZIEGLER and HARMSEN 1969; PHILLIPS and FORREST 1980). The presence of pteridines in other tissues and stages is sometimes indicated by slight tissue pigmentation. Their functions, in general, are not well understood. In Drosophila and other organisms, however, derivatives of biopterin act as cofactors for various enzymes, including phenylalanine hydroxylase and the group of enzymes known as the molybdenum hydroxylases which includes xanthine dehydrogenase, aldehyde oxidase and pyridoxal oxidase (SCRIVER and CLOW 1980; RAJAGOPALAN, JOHNSON and HAINLINE 1982; WAHL *et al.* 1982).

Primarily through the initial and continuing efforts of GENE BROWN and his collaborators, a number of the enzyme reactions necessary for pteridine biosynthesis in Drosophila have been elucidated, and some of the enzymes have been purified (FAN and BROWN 1976, 1979; KRIVI and BROWN 1979; WIEDERNECHT, PATON and BROWN 1981; UNNASCH and BROWN 1982). The first reaction in pteridine biosynthesis is the conversion of guanosine triphosphate (GTP) to dihydroneopterin triphosphate with the release of formic acid. It is catalyzed by the enzyme GTP cyclohydrolase (GTP CH) (EC 3.5.4.16, GTP 7, 8-8,9-dihydrolase) (BROWN *et al.* 1979). In Drosophila, stage-specific changes in GTP CH activity correspond to changes in pterin levels (FAN *et al.* 1976; EVANS and HOWELLS 1978). The major portion of GTP CH activity occurs in a very short-lived peak that reaches a maximum level at, or shortly prior to, eclosion. The activity then decreases rapidly and, within 48 hr after eclosion, GTP CH activity is less than 20% of its maximum activity. Approximately 80–90% of the total enzyme activity in young adults resides in the head (FAN *et al.* 1976; EVANS and HOWELLS 1978). A small transient peak of activity also occurs with the onset of pupation. EVANS and HOWELLS (1978) found that mutations in the prune locus (*pn*, 1-0.8) and raspberry locus (*ras*, 1-32.8) cause a reduction in GTP CH activity in the heads of young adults, whereas the activity in other tissues is normal or greater than normal. They also observed that GTP CH activity in *ras* and *pn* mutant/wild-type heterozygotes was normal. Since the loci do not show a gene dosage effect on GTP CH activity, these authors speculate that pn^+ and ras^+ participate in the developmental regulation of GTP CH rather than being GTP CH structural genes.

We have begun a genetic study of pteridine biosynthesis with this first reaction in the pathway. We expected regulation at the first step to be particularly important in the production of pteridines and thus provide fertile ground for an investigation of mechanisms controlling gene expression. The observations of EVANS and HOWELLS (1978) offer a first suggestion that our expectations would be met. The work of BROWN and collaborators in Drosophila and in *E. coli* (BURG and BROWN 1968; YIM and BROWN 1976, FAN and BROWN 1979) provided preliminary information about the biochemical characteristics of GTP CH and a highly sensitive assay method. Our first task was to define, in a more complete way, the genetic control of GTP CH expression, including an attempt to identify loci in addition to *pn* and *ras* that participate in GTP CH function. Given that GTP CH activity is essential to the production of pteridine cofactors, we expected that severe reduction in enzyme activity would

result in a lethal phenotype. However, since the reaction is the first in the pathway and since it is required for the production of all other pteridines, it seemed likely that even a slight reduction might result in viable individuals with mutant eye color. Therefore, we began our study with a survey of eye color mutants having demonstrated or probable pteridine defects. We report here the details of the survey, which include the identification of several additional loci affecting GTP CH activity and the verification of the results obtained by EVANS and HOWELLS (1978). We also describe the characteristics of mutations in these loci. As one might expect, considering the bifunctionality of the pathway and the position of the GTP CH reaction as essentially a bridge between purine metabolism and the pteridine pathway, the genetics of GTP CH expression are rather complex. The system responds to mutational perturbation with a very wide range of phenotypic effects that provide sensitive indicators of changes in the function of the enzyme.

MATERIALS AND METHODS

Strains: All strains were maintained in half-pint bottles on standard medium at 25°. When progeny were to be used for comparative enzyme assays, 30 females and 15 males were placed on fresh medium for 2–3 days, if strains were homozygous viable. For balanced lethal stocks, the numbers of parents were doubled. Extra yeast was added to the bottles when the parents were removed and several additional times before progeny were collected.

The strains used in the experiments are described by LINDSLEY and GRELL (1968) except for the following: Pu^{r331} was derived from a population collected in DeKalb County, Georgia, and stocks 224 and 265 are eye color mutants collected in Clarke County, Georgia. They were generously provided by VICTORIA FINNERTY and CYNTHIA WARNER. The *X*-linked eye color mutation, cinnamon, is described by BAKER (1973), and the stock $cin^{9}y$ was a gift from JOHN WILLIAMSON. The mutation, *vin*, is described by ANXOLABEHERE and PERIQUET (1973). The γ-ray-induced inversion strain *In(2LR)R-3;* $SD^{R\text{-}1}$ *1t* $Pu^{L}/Cy0$ was generated by TERRENCE LYTTLE. *T(Y;2)JL-11* is a rearrangement line generated by radiation-induced secondary translocation of *T(Y;2)L141* in which Y^{S} carries chromosome *2R* segment 56F-57F (T. LYTTLE unpublished data). Pu^{r1} was discovered by PHILIP IVES and sent to us by LORING CRAYMER.

Enzyme assays: The methods used for the assay of GTP CH are modifications of the method described by FAN and BROWN (1976). The assay is based on the enzymatic release of ^{14}C-formic acid from [8-^{14}C]GTP. For all assays of adults, individuals were collected less than 8 hr after eclosion from cultures that had been eclosing not more than 3 days. For assays of prepupae, white pupae were removed from the sides of culture bottles at hourly intervals. Unless otherwise noted, samples were frozen at −75° for 3 wk or less prior to assay. We have ascertained that the enzyme activity of samples frozen under these conditions is not notably different from the activities in unfrozen samples.

For whole fly assays, 0.15 g of adult flies were homogenized in 2 ml of cold 0.1 M Tris-HCl, pH 8.75, containing 10 mM EDTA, sodium salt, and 5 mM NaN_3. The homogenates were centrifuged at 20,000 rpm in a Sorvall centrifuge for 20 min. Fifteen milligrams of well-rinsed activated charcoal were added with mixing to the supernatants. After another centrifugation, the extracts were filtered through type HA Millipore filters, pore size 0.45 μm, and assayed. Reaction mixtures contained, in a total volume of 1.1 ml, 0.1 M Tris-HCl, pH 8.75, 10 mM EDTA, sodium salt, 5 mM NaN_3, 200 μM GTP, and 0.4 ml of extract. The GTP substrate was a mixture of 7 μl of [8-^{14}C]GTP (Amersham, specific activity 55 mCi/mmol, 50 μCi/ml) and 8.75 μl of 10 mM unlabeled GTP (Sigma). Each assay mixture was incubated at 42° for 10 min. The reaction was stopped with 0.1 ml of 1 N formic acid. After the addition of 150 mg of charcoal and 0.5 ml of distilled water, the mixtures were placed on a shaker table for 30 min. The suspensions and an additional rinse of 0.25 ml of distilled water were filtered through nonabsorbent cotton; 0.5 ml of the filtrate was mixed with counting cocktail, and radioactivity was determined by liquid scintillation counting.

Background counts obtained from a nonincubated sample were subtracted from total counts for each assay.

The assay was further modified individually for the assay of GTP CH in prepupae and in separated adult heads and bodies. Each version samples GTP CH within a linear range of activity and is optimal for the particular tissue type. Thirty individuals were used for each assay. Adult heads and bodies were first separated by microscalpel and then were homogenized in 200 μl of 0.1 M Tris-HCl, pH 8.70. For prepupal assays, the homogenization solution also contained 10 mM NaN_3, 20 mM EDTA, and 60 mg/ml of rinsed activated charcoal. For adult head assays, 2 mM NaN_3, 10 mM EDTA, and 1.0 mg/ml of rinsed activated charcoal were included. For adult body assays, 5 mM EDTA was included. The homogenates were centrifuged for 2 min in a Beckman-Eppendorf Airfuge. Forty-seven microliters of each supernatant were mixed with 3 μl of GTP reaction mix containing 50 μM [8-^{14}C]GTP and 50 μM unlabeled GTP. Assays were carried out as previously described with a 15-min incubation period for adult head and prepupal extracts and a 20-min incubation for adult body extracts. Enzyme activities are reported as nanomoles of formate released per hour per mg of protein.

Protein determinations: Protein concentrations in the extracts were determined by the method of LOWRY *et al.* (1951).

RESULTS

Survey of pteridine-defective mutants: We first asked whether or not GTP CH activity was altered in any mutants that had pteridine defects. We included both mutants for which pteridine contents had been determined and mutants whose eye color phenotypes suggested pteridine defects (*i.e.*, colors in the brown or purple range). We also assayed certain strains carrying mutations that interact in some manner with pteridine genes or enzymes, such as Killer of prune (*K-pn*) and low xanthine dehydrogenase (*lxd*). This collection of mutants has been derived from many sources, so we were aware from the outset that any activity variation detected would not necessarily be associated with the eye color mutation in question. Any GTP CH activity variants that we identified would simply be source material for further experiments.

In all experiments, Canton S or Oregon R wild-type strains were assayed simultaneously with mutant strains. These two wild-type strains have virtually identical activity levels. The results we report here were obtained from assays of either whole adult flies or adult heads. In most cases, both types of assays were performed and activities relative to the wild-type standard were very similar. The assays in this survey were performed over a long period of time with several variations in conditions. Although our internal controls assured us that relative activities did not vary greatly as a result, the activities in nanomoles of formate generated are not directly comparable. Therefore, for this survey, only relative activities are reported. All subsequent experiments were then performed under conditions that permitted comparison of specific activities directly.

Table 1 summarizes the results of the survey. We obtained a wide range of relative activities from white which had 181% of wild-type activity to about 20% of normal for several prune alleles. Several mutant strains in addition to those carrying *pn* alleles had reduced GTP CH activities, a condition that we arbitrarily define as being 70% or less of wild-type activity. We have concentrated on these mutants in all subsequent studies. All alleles of *ras* also show a reduction in enzyme activities. Similar results for *pn* and *ras* mutants had

TABLE 1

GTP cyclohydrolase activity survey

Mutation	Genotype	Map location[a]	Activity (relative to wild type)[b]
bordeaux	*bo/bo*	1–12.5	1.15 (1.06–1.22) [4]
brown	*bw/bw*	2–104.5	1.15 (1.06–1.28) [6]
	cn bw/cn bw	2–104.5	1.16 (1.01–1.42) [3]
burgundy	*bur fs2.1/Cy*	2–55.7	0.92 (0.80–1.04) [2]
carmine	*cm/cm*	1–18.9	0.99 (0.91–1.11) [6]
carnation	*car/car*	1–62.5	1.00 (0.86–1.16) [4]
chocolate	*sc cho/sc cho*	1–5.4	0.85 (0.72–0.96) [5]
cinnamon[9]	cin^9y/cin^9y	1–0.0[c]	0.99 (0.79–1.18) [3]
claret	*ca/ca*	3–100.7	1.22 (1.09–1.34) [4]
clot	*cl/cl*	2–16.5	0.76 (0.69–0.88) [10]
dark red brown	*drb/drb*	3–47.7	0.93 (0.83–1.03) [5]
deep orange	*dor/dor*	1–0.3	1.19 (0.99–1.39) [6]
garnet	*g/g*	1–44.4	1.06 (1.04–1.08) [3]
Henna[r3]	$Hn^{r3}sr/Hn^{r3}sr$	3–23.0	1.24 (1.18–1.30) [2]
Killer of prune	*ca K-pn/ca K-pn*	3–102.9	1.17 (1.03–1.41) [5]
lightoid	*ltd/ltd*	2–56	1.13 (1.01–1.24) [3]
low xanthine dehydrogenase	*lxd/lxd*	3–33	1.06 (0.92–1.19) [2]
mahogany	*mah/mah*	3–88.0	1.01 (0.94–1.07) [4]
maroon	*ma fl/ma fl*	3–49.7	0.96 (0.83–1.14) [4]
maroon-like[Q]	$y\ v\ ma\text{-}l^Q/y\ v\ ma\text{-}l^Q$	1–64.8	1.02 (1.00–1.04) [2]
orange[49H]	or^{49H}/or^{49H}	2–107.2	1.42 (1.41–1.42) [2]
pink	*p/p*	3–48.0	1.06 (0.99–1.14) [5]
prune	*y pn/y pn*	1–0.8	0.33 (0.28–0.39) [8]
prune[2]	pn^2/pn^2	1–0.8	0.21 (0.09–0.24) [5]
prune[3]	pn^3/pn^3	1–0.8	0.22 (0.21–0.23) [2]
prune[54C]	$y\ pn^{54C}spl/y\ pn^{54C}spl$	1–0.8	0.18 (0.17–0.20) [5]
Punch[2]	$Pu^2/SMl,al^2\ Cy\ cn^2\ sp^2$	2–97	0.33 (0.31–0.35) [6]
Punch[r331]	$cn^{331}\ Pu^{r331}/cn^{331}\ Pu^{r331}$	2–97[d]	0.30 (0.20–0.41) [6]
purple	*pr/pr*	2–54.5	1.01 (1.00–1.07) [5]
purploid	*pd/pd*	2–106.4	0.90 (0.79–1.07) [6]
Purploider	*px pd; Pdr H Dp(2;3)P/Pdr (2;3)*	3–46	1.08 (0.90–1.34) [4]
raspberry	*ras dy/ras dy*	1–32.8	0.45 (0.42–0.52) [6]
raspberry[2]	ras^2/ras^2	1–32.8	0.27 (0.25–0.31) [3]
raspberry[3]	ras^3m/ras^3m	1–32.8	0.79 (0.68–0.84) [4]
red Malpighian tubules	*red/red*	3–53.6	0.62 (0.50–0.74) [10]
rose[2]	rs^2/rs^2	3–35.0	0.52 (0.41–0.68) [5]
rosy[8]	ry^8/ry^8	3–52.0	0.80 (0.74–0.93) [5]
ruby	*rb/rb*	1–7.5	1.00 (0.93–1.18) [4]
sepia	*se/se*	3–46.0	1.07 (0.94–1.16) [7]
safranin[2]	sf^2/sf^2	2–71.5	1.05 (0.95–1.10) [6]
vin	*vin/vin*	3–36.3[e]	0.99 (0.83–1.23) [6]
white	*w/w*	1–1.15	1.81 (1.62–2.00) [2]
zeste	*z/z*	1–1.0	1.56 (1.33–1.69) [4]
	z/y	1–1.0	1.02 (0.97–1.11) [3]
stock 224	*224/224*	?[f]	0.94 (0.82–1.03) [4]
stock 265	*265/265*	?[f]	0.93 (0.86–1.02) [3]

[a] Map locations are reported in LINDSLEY and GRELL (1968) except where otherwise noted.

[b] Enzyme activities are relative to Canton S or to Oregon R, which have nearly identical GTP cyclohydrolase activities. The numbers in parentheses show the range of relative activities obtained and those in brackets, the number of independent tissue preparations and assays.

[c] Map location taken from O'BRIEN and MACINTYRE (1978).

[d] Discovered by VICTORIA FINNERTY.

[e] Mutations and map location reported in ANXOLABEHERE and PERIQUET (1973).

[f] Eye color mutations from collection of VICTORIA FINNERTY. Map locations not yet available.

been observed earlier by EVANS and HOWELLS (1978). Other strains showing reductions in activity were Punch^2 (Pu^2), a second chromosome mutation, rose^2 (rs^2), and red Malpighian tubules (*red*), third chromosome mutations, and what was, at the time, an unmapped eye color mutation designated by stock number, 331. We subsequently ascertained that stock 331 carried an allele of cinnabar and a recessive allele of Punch. We determined, by recombination analysis, that the reduced GTP CH activity phenotype was associated with the *Pu* mutation. In Table 1 the strain is designated as Pu^{r331}.

Characterization of reduced activity mutants: We determined the enzyme activity levels in prepupae and in adult head and body segments from each of the mutants having reduced activity (Table 2) and from mutant/wild-type heterozygotes (Table 3). Pu^2 is not included in Table 2 because it is homozygous lethal. With the exception of Pu^{r331}, which has a slightly lower than normal prepupal activity, the homozygous mutants do not show a reduction of GTP CH activity in the adult bodies or prepupae, and most actually have greater prepupal activities than the Canton S level.

Except in the case of Pu^2, the prepupal and adult body tissues of mutant/wild-type heterozygotes possess at least normal or nearly normal GTP CH levels (Table 3). The adult head activities of each heterozygote are also normal. Pu^2/*Canton S*, on the other hand, demonstrates severe and equivalent reductions of GTP CH activity in each case.

Effects of Pu *region dosage on GTP cyclohydrolase activity:* Pu^{r331} and Pu^2 appear to be allelic. Both arose spontaneously as wild population isolates, and both are cytologically normal. There are, however, important differences in their phenotypes. Pu^{r331} is recessive, homozygous viable and, in a heterozygous state with Pu^+, has normal enzyme activity. Pu^2 has a dominant nonvariegating eye color, recessive lethal phenotype, and is the only mutant in our survey demonstrating effects on the GTP CH activity in the heterozygous condition. The Pu^2 homozygote dies in an embryonic stage of development, so we are unable to compare homozygote *vs.* heterozygote activities at any stage. We asked instead whether varying doses of wild-type Punch alleles would affect GTP CH activity. Individuals having one dose of the Punch region were obtained from a deficiency strain that we made by γ-irradiation, *Df(2R)*Pu^{rF36}. Chromosome *2R* segments 57C1-57C8, inclusive, are deleted in this mutant. Hyperploid male segregants from the translocation strain, *T(Y;2)JL-11*, were used to test the effects of three doses of the Punch region. The *Y* chromosome in this strain carries chromosome *2R* region 56F-57F which includes the Punch region (T. LYTTLE, unpublished data). The source of euploid individuals was a strain carrying the recessive markers *cn*, *a*, *px* and *sp*. It is the parent strain of *Df(2R)*Pu^{rF36}. The results of the experiment are presented in Table 4. When males from each genotype were assayed for GTP CH activity, a strict correlation between the number of Pu^+ genes and the level of GTP CH in each class was observed. The eye color of the deficiency/Pu^+ individuals is similar to that of *cn* Pu^2/*cn* Pu^+ for the first 1–3 hr after eclosion. After that time, the eye color is indistinguishable from that of *cn*/*cn* individuals.

GTP CH in the Pu^2 *mutant:* The effect of the Pu^2 mutation on GTP CH activity is considerably more severe than that of a deletion of the *Pu* region.

TABLE 2

The tissue distribution of GTP cyclohydrolase activity in homozygous reduced activity mutants

	Prepupae	Adult body	Adult head
Canton S	0.93 ± 0.13	0.64 ± 0.05	40.39 ± 2.60
pn	1.74 ± 0.43 (1.87)	0.70 ± 0.11 (1.09)	16.01 ± 1.61 (0.40)
pn^2	2.54 ± 0.29 (2.73)	0.51 ± 0.08 (0.80)	8.44 ± 0.98 (0.21)
pn^3	2.20 ± 0.09 (2.37)	0.51 ± 0.09 (0.80)	8.90 ± 0.04 (0.22)
ras	1.25 ± 0.22 (1.34)	0.65 ± 0.06 (1.02)	18.22 ± 1.76 (0.45)
ras^2	2.29 ± 0.38 (2.86)	0.56 ± 0.07 (0.88)	10.86 ± 1.42 (0.27)
ras^3	1.25 ± 0.25 (1.34)	0.64 ± 0.13 (1.00)	31.65 ± 2.95 (0.78)
rs^2	1.50 ± 0.22 (1.61)	0.55 ± 0.03 (0.86)	23.26 ± 2.40 (0.58)
red	1.85 ± 0.21 (1.99)	0.52 ± 0.04 (0.81)	27.59 ± 0.23 (0.68)
Pu^{r331}	0.70 ± 0.09 (0.75)	0.59 ± 0.12 (0.92)	10.09 ± 0.58 (0.25)

Activities are reported as nanomoles of formate released per hour per milligram of protein in crude extracts and are the means of three to six independent assays ± S.D. The numbers in parentheses are activities relative to the wild-type strain, Canton S.

TABLE 3

GTP cyclohydrolase activity in reduced activity mutant/wild-type heterozygotes

Heterozygote	Prepupae	Adult body	Adult head
pn/Canton S	0.75 ± 0.10 (0.81)	0.54 ± 0.06 (0.84)	43.04 ± 4.82 (1.07)
ras/Canton S	0.75 ± 0.07 (0.81)	0.57 ± 0.12 (0.89)	41.83 ± 0.29 (1.04)
rs^2/Canton S	1.67 ± 0.03 (1.80)	0.45 ± 0.07 (0.75)	42.63 ± 0.16 (1.06)
red/Canton S	1.29 ± 0.19 (1.39)	0.54 ± 0.13 (0.84)	37.30 ± 3.10 (0.92)
Pu^{r331}/Canton S	0.92 ± 0.13 (0.99)	0.60 ± 0.06 (0.94)	43.70 ± 2.23 (1.08)
Pu^2/Canton S	0.18 ± 0.04 (0.19)	0.22 ± 0.02 (0.34)	13.04 ± 0.63 (0.32)

Activities are reported as nanomoles of formate released per hour per milligram of protein in crude extracts and are the means of three to six independent assays ± S.D. The numbers in parentheses are activities relative to Canton S activities: 0.93 ± 0.13 = prepupae; 0.64 ± 0.05 = adult body; 40.39 ± 2.60 = adult head.

TABLE 4

GTP cyclohydrolase activity with increasing doses of Pu^+

1 dose	*Df(2R)*Pu^{rF36}*,cn* Pu^{rF36} *a px sp/cn a px sp*	Head 12.87 ± 1.08 [0.46] Body 0.26 ± 0.04 [0.47]
2 doses	*cn a px sp/cn a px sp*	Head 27.99 ± 0.54 [1.00] Body 0.55 ± 0.10 [1.00]
3 doses	*T(Y → 2)JL-11,*Pu^+*/cn bw/cn a px sp*	Head 43.47 ± 0.02 [1.55] Body 0.92 ± 0.09 [1.67]

Activities are reported as nanomoles of formate released per hour per milligram of protein in crude extracts. Each activity is the mean of four to five independent assays ± S.D. The numbers in parentheses are GTP CH activities relative to the two-dose activity.

In order to examine the properties of Pu^2 further, we carried out crosses designed to test the consequences of varying the number of Pu^2 and Pu^+ alleles on viability and on GTP CH activity. Two translocation strains that carry the Pu^+ region on the *Y* chromosome were utilized. One, *T(Y;2)JL-11*, was de-

scribed in the previous section. Another, *T(Y;2)R31,* carries chromosome *2R* sections 57A-58A on the *Y* chromosome.

The first cross involving *T(Y;2)JL-11* produces four classes of males as illustrated in Figure 1. A second cross using *T(Y;2)R31, 57A-58A; Df(2R)57A-58A/SM1* produces only three classes of males since the progeny class *Dp(Y;2)R31, Pu^+; SM1/SM1* is lethal. The other progeny classes are comparable to those of the previous experiment. Assays of two and three Pu^+ dose classes showed the expected levels of GTP CH activity (Table 5). The fact that one of the Pu^+ genes in the two-dose class had been moved to the *Y* chromosome had little or no effect on the enzyme activity or on the eye color phenotype. The Pu^2/Pu^+ class has severely reduced head and body activities and a mutant eye color indistinguishable from that observed when the homologous Pu^+ gene is in its normal second chromosome position. The class of progeny having two Pu^+ genes and Pu^2 shows a slight eye color effect in very young adults, but it very rapidly attains wild-type pigmentation within 1–2 hr of eclosion and after that time cannot be phenotypically distinguished from Pu^+/Pu^+. The GTP CH activity of this class is very close to wild-type levels of activity.

Pu^2 is homozygous lethal and is lethal in combination with $Df(2R)Pu^{rF36}$. We crossed males of the genotype, *Dp(Y;2)JL-11, 56F-57F;* $Pu^2/SM1$ to $Pu^2/SM1$ females to determine whether a single dose of Pu^+ could rescue Pu^2/Pu^2 progeny. *Dp(Y;2)JL-11 56F-57F;* Pu^2/Pu^2 progeny do not survive. We conclude from all of these experiments that Pu^2 appears to have an antimorphic effect on GTP CH.

Characteristics of other Punch alleles: Several other alleles of Punch were available, so we next examined their effects on GTP CH. Table 6 summarizes some relevant information concerning the existing *Pu* alleles as well as several that we subsequently generated. The results of GTP CH assays of these mutants are presented in Table 7. There are several other dominant alleles of Punch. Those we examined were Pu^{Gr}, Pu^L and Pu^W. Unlike the cytologically normal Pu^2 allele, each of these mutations is associated with a chromosomal rearrangement involving a heterochromatic breakpoint and another in or near region *57C* on chromosome *2R* where the *Pu* locus is localized, and each has a dominant, variegated eye color. In addition to dominant eye color effects, they share with Pu^2 the characteristic of recessive lethality. All pairwise combinations of these alleles are also lethal. In a heterozygous condition with a normal Pu^+ allele, each of these mutations has a severe effect on GTP CH activity in adult tissues. The prepupal activity is also diminished, although not to the same extent. In each case, the prepupal activity is greater than 50% of the normal activity.

Another recessive allele to *Pu*, Pu^{r1} was also available. Like Pu^{r331} it is a homozygous viable and fertile spontaneous mutation (Table 6). The eye color of homozygous Pu^{r1} is more extreme than that of the dominant allele heterozygotes or Pu^{r331} and is somewhat darker than the eye color of the mutant, purple. The double mutant *cn* Pu^{r1} has very pale orange, nearly white, eyes in young adults and gradually acquires more orange pigmentation as the animal ages. The color remains, however, a dilute orange. The Pu^{r1}/Pu^+ heterozygote

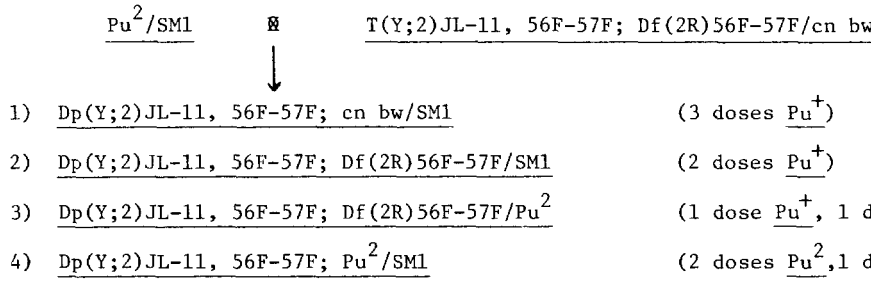

FIGURE 1.—Genotypic class of male progeny derived from the mating of *Pu²/SM1* females to *T(Y;2)JL-11* males.

TABLE 5

Effects of Pu² *on* Pu⁺ *GTP CH activity*

(3 doses Pu^+)		(2 doses Pu^+)	(1 dose Pu^+, 1 dose Pu^2)	(2 doses Pu^+, 1 dose Pu^2)
Dp(Y;2)JL-11, Pu⁺; cn Pu⁺ bw/ SM1, Pu⁺[a]		*Dp(Y;2)JL-11, Pu⁺; Df(2R)Pu/SM1, Pu⁺*	*Dp(Y;2)JL-11, Pu⁺/ Df(2R)Pu/Pu²*	*Dp(Y;2)JL-11, Pu⁺/cn Pu⁺ bw/Pu²*
Head	50.82 ± 8.72 [1.68][b]	30.10 ± 7.57 [1.00]	9.70 ± 1.09 [0.32]	26.51 ± 4.46 [0.88]
Body	1.33 ± 0.07 [1.64]	0.81 ± 0.04 [1.00]	0.21 ± 0.08 [0.26]	0.69 ± 0.04 [0.85]
Dp(Y;2)R31, Pu⁺; SM1/SM1		*Dp(Y;2)R31, Pu⁺; Df(2R)Pu/SM1, Pu⁺*	*Dp(Y;2)R31, Pu⁺/ Df(2R)Pu/Pu²*	*Dp(Y;2)R31, Pu⁺/SM1, Pu⁺/Pu²*
Head	Lethal	33.37 ± 4.62 [1.00]	9.15 ± 0.33 [0.27]	27.06 ± 4.72 [0.81]
Body		0.69 ± 0.06 [1.00]	0.20 ± 0.03 [0.29]	0.57 ± 0.03 [0.83]

Activities are presented as nanomoles of formate per hour per milligram protein in crude extracts. The numbers were calculated from three independent assays of each class and are presented as the means ± S.D.

[a] The crosses used to generate the male progeny classes were: *cn Pu²/SM1 × T(Y;2)JL-11, 56F–57F; Df(2R)56F–57F/cn bw* and *cn Pu²/SM1 × T(Y;2)R31, 57A–58A; Df(2R)57A–58A/SM1.*

[b] The numbers in brackets are activities relative to the activity of two doses of Pu^+ in each cross. *Pu²/SM1* and Canton S males were assayed at the same time. The activities were: *Pu²/SM1* = 11.84 ± 2.26 (head) and 0.22 ± 0.03 (body). Canton S = 38.31 ± 0.40 (head) and 0.50 ± 0.02 (body).

has a slightly purple eye, clearly distinguishable from wild type, in very young adults. Within a few hours after eclosion, however, the eye phenotype becomes normal. We were somewhat surprised to observe that Pu^{r1} is also associated with a chromosomal rearrangement. The rearrangement in this case is a small inversion in the *57C-E* region of *2R*. The proximal breakpoint of this inversion is located near breakpoints associated with other *Pu* rearrangements. The breakpoints in Pu^{r1} do not, however, involve heterochromatin. (A detailed cytological analysis will be presented in a future publication.) Pu^{r1} is viable in combination with all of the dominant alleles.

The head-specific GTP CH of homozygous Pu^{r1} is severely reduced, having only 5% of the wild-type levels of activity (Table 7). Unlike activity levels in Pu^{r331}/Pu^+ heterozygotes, GTP CH activity in the head tissues of Pu^{r1}/Pu^+ heterozygotes is precisely intermediate between that of Pu^{r1} and wild type.

TABLE 6

Punch alleles

Allele	Origin	Genotype	Phenotype
Pu^2	Spontaneous[a]	Pu^2	Dominant eye color; homozygous lethal
Pu^{Gr}	X-ray[b]	$T(2;3)Pu^{Gr}$, Pu^{Gr}	Dominant, variegated eye color; homozygous lethal
Pu^W	X-ray[c]	$T(2;3)Pu^W$, Pu^W	Dominant, variegated eye color; homozygous lethal
Pu^L	γ-ray[d]	$In(2LR)R\text{-}3$, $SD^{R\text{-}1}lt\ Pu^L$	Dominant, variegated eye color; homozygous lethal
Pu^{rF36}	γ-ray[e]	$DF(2R)Pu^{rF36}$, $dp\ cn\ Pu^{rF36}\ a\ px\ sp$	Recessive eye color; homozygous lethal
Pu^{rS47}	EMS[e]	$dp\ cn\ Pu^{rS47}a\ px\ sp$	Recessive eye color; homozygous lethal
Pu^{rP21}	EMS[e]	$dp\ cn\ Pu^{rP21}a\ px\ sp$	Recessive eye color; homozygous lethal
Pu^{rP1}	EMS[e]	$Tp(2LR)Pu^{rP1}$, $dp\ cn\ Pu^{rP1}\ a\ px\ sp$	Recessive eye color; homozygous lethal
Pu^{rP42}	EMS[e]	$dp\ cn\ Pu^{rP42}\ a\ px\ sp$	Recessive eye color; homozygous lethal
Pu^{rP30}	EMS[e]	$dp\ cn\ Pu^{rP30}\ a\ px\ sp$	Recessive eye color; homozygous lethal
Pu^{rP43}	EMS[e]	$dp\ cn\ pu^{rP43}\ a\ px\ sp$	Recessive eye color; homozygous lethal
Pu^{rI9}	EMS[e]	$dp\ cn\ Pu^{rI9}\ a\ px\ sp$	Recessive eye color; homozygous lethal
Pu^{rP11}	EMS[e]	$dp\ cn\ Pu^{rP11}\ a\ px\ sp$	Recessive eye color; homozygous lethal
Pu^{r1}	Spontaneous[f]	$In(2R)Pu^{r1}$, Pu^{r1}	Recessive eye color; homozygous viable
Pu^{r331}	Spontaneous[g]	$cn^{331}\ Pu^{r331}$	Recessive eye color; homozygous viable
Pu^{rX17}	EMS[e]	$dp\ cn\ Pu^{rX17}\ a\ px\ sp$	Recessive eye color; homozygous viable
Pu^{rAA4}	EMS[e]	$dp\ cn\ Pu^{rAA4}\ a\ px\ sp$	Recessive eye color; homozygous viable
Pu^{rZ8}	EMS[e]	$dp\ cn\ Pu^{rZ8}\ a\ px\ sp$	Recessive eye color; homozygous viable

[a] Discovered by E. H. GRELL (LINDSLEY and GRELL 1968; E. H. GRELL, personal communication).
[b] Discovered by MULLER (LINDSLEY and GRELL 1968).
[c] Discovered by E. B. LEWIS (LINDSLEY and GRELL 1968).
[d] Discovered by T. LYTTLE (T. LYTTLE, personal communication).
[e] Generated in this laboratory.
[f] Discovered by P. IVES (L. CRAYMER, personal communication).
[g] Discovered by V. FINNERTY (V. FINNERTY, personal communication).

The adult body activities of the homozygote and the heterozygote are normal. The prepupal activity appears to be affected, but the effect is slight compared with the adult head-specific effect. Developmental studies show that the severe reduction in head-specific activity cannot be attributed to a temporal displacement of the major peak of enzyme activity and that the developmental expres-

TABLE 7

GTP cyclohydrolase activity in Pu *mutants*

Allele	Prepupae	Adult body	Adult head
Pu^+ (Canton S)	0.93 ± 0.13	0.64 ± 0.05	40.39 ± 2.60
Pu^+ *(dp cn a px sp)*	1.31 ± 0.19 [1.41]	0.69 ± 0.12 [1.08]	36.02 ± 1.84 [0.89]
Pu^2/Pu^+	0.18 ± 0.04 [0.19]	0.22 ± 0.02 [0.34]	13.04 ± 0.63 [0.32]
Pu^{Gr}/Pu^+	0.58 ± 0.04 [0.62]	0.29 ± 0.04 [0.45]	13.63 ± 4.08 [0.34]
Pu^W/Pu^+	0.56 ± 0.16 [0.60]	0.10 ± 0.03 [0.16]	3.17 ± 0.54 [0.08]
Pu^L/Pu^+	0.52 ± 0.10 [0.56]	0.12 ± 0.03 [0.19]	10.28 ± 1.71 [0.25]
Pu^{rF36}/Pu^+	0.48 ± 0.04 [0.52]	0.30 ± 0.04 [0.47]	20.86 ± 2.74 [0.52]
Pu^{rS47}/Pu^+	0.48 ± 0.07 [0.52]	0.35 ± 0.08 [0.55]	27.18 ± 2.70 [0.67]
Pu^{rP21}/Pu^+	0.48 ± 0.08 [0.52]	0.31 ± 0.07 [0.48]	25.57 ± 3.50 [0.63]
Pu^{rP1}/Pu^+	0.45 ± 0.03 [0.48]	0.18 ± 0.03 [0.28]	21.34 ± 2.29 [0.53]
Pu^{rP42}/Pu^+	0.78 ± 0.11 [0.84]	0.40 ± 0.08 [0.63]	27.34 ± 3.33 [0.68]
Pu^{rP30}/Pu^+	0.43 ± 0.05 [0.46]	0.29 ± 0.06 [0.45]	23.68 ± 2.76 [0.58]
Pu^{rP43}/Pu^+	0.90 ± 0.12 [0.97]	0.51 ± 0.04 [0.80]	33.29 ± 1.89 [0.82]
Pu^{rI9}/Pu^+	0.63 ± 0.11 [0.67]	0.19 ± 0.02 [0.30]	25.68 ± 1.86 [0.64]
Pu^{rP11}/Pu^+	0.73 ± 0.12 [0.75]	0.39 ± 0.03 [0.61]	24.57 ± 3.35 [0.61]
Pu^{r1}/Pu^{r1}	0.57 ± 0.14 [0.61]	0.58 ± 0.05 [0.91]	1.91 ± 0.45 [0.05]
Pu^{r1}/Pu^+	0.74 ± 0.12 [0.80]	0.64 ± 0.10 [1.00]	22.58 ± 2.39 [0.56]
Pu^{r331}/Pu^{r331}	0.70 ± 0.09 [0.75]	0.59 ± 0.12 [0.92]	10.09 ± 0.58 [0.25]
Pu^{r331}/Pu^+	0.92 ± 0.13 [0.99]	0.60 ± 0.06 [0.94]	43.70 ± 2.23 [1.08]
Pu^{rX17}/Pu^{rX17}	0.86 ± 0.06 [0.94]	0.51 ± 0.03 [0.80]	2.35 ± 0.16 [0.06]
Pu^{rX17}/Pu^+	1.11 ± 0.08 [1.19]	0.65 ± 0.03 [1.02]	24.38 ± 3.58 [0.60]
Pu^{rAA4}/Pu^{rAA4}	0.72 ± 0.12 [0.77]	0.26 ± 0.09 [0.41]	3.35 ± 0.37 [0.08]
Pu^{rAA4}/Pu^+	0.82 ± 0.14 [0.88]	0.52 ± 0.09 [0.81]	26.25 ± 2.49 [0.65]
Pu^{rZ8}/Pu^{rZ8}	0.77 ± 0.11 [0.83]	0.46 ± 0.07 [0.72]	2.09 ± 0.32 [0.05]
Pu^{rZ8}/Pu^+	0.95 ± 0.14 [1.02]	0.53 ± 0.07 [0.88]	22.71 ± 2.55 [0.56]

Activities are presented as nanomoles of formate per hour per milligram of protein in crude extracts. The numbers are the means of four to six independent assays ± S.D.

sion of the body-specific activity is normal (W. J. MACKAY and J. M. O'DONNELL, unpublished observation).

Generation and characterization of new Punch alleles: We wished to know what effect point mutations in a defined *Pu* locus would have on GTP CH activity. Hence, we turned to mutagenesis with ethyl methanesulfonate (EMS), following the standard LEWIS and BACHER (1968) method. Our initial experiments utilized a simple F_1 screen of treated males from a marked second chromosome strain, *dp cn a px sp.* Testor females were Pu^{r1} *a px sp.* We used Pu^{r1} in this screen since it survives in combination with all of the dominant *Pu* mutations. Any new, severe mutations should also survive in combination with this allele. Furthermore, Pu^{r1} itself shows a slight, transient eye color effect when heterozygous with Pu^+. Therefore, we anticipated the detection of very leaky mutations as well. We have screened about 70,000 progeny and have obtained 26 new, fertile, *Pu* mutations. All of these mutations are recessive if the phenotype is monitored at 6 hr or more after eclosion. Before that time, there is a wide variation in eye color effects from clearly mutant to indistinguishable from wild type. Most of the new mutations are homozygous lethal. All homozygous lethal mutations are also lethal in combination with $Df(2R)Pu^{rF36}$ and

are lethal or semilethal in all *inter se* allele combinations. Survivors of the semilethal heteroallelic combinations have combination-specific pigmentation levels, but all are severely mutant in eye color. A complete complementation analysis of all *Pu* mutations will be presented in a subsequent report (W. J. MACKAY and J. M. O'DONNELL, unpublished data). The activities of representative samples of the new mutants are shown in Table 7. In most cases, adult head and body and prepupal activities are affected to equivalent extents. Occasionally, we observe a more severe effect in the adult body as in the case of Pu^{rP1} and Pu^{rI9}. The amount of eye pigmentation in *cn* Pu^{r1}/*cn* Pu^{EMS} heterozygotes correlates quite well with the observed head-specific GTP CH activity.

We have obtained three EMS-induced *Pu* mutations that are homozygous viable. Each has a strong eye color defect but does make a small amount of red pigment. In a *cn* background, each of these mutants is similar but distinct, and each is distinguishable from *cn* Pu^{r1}. The homozygous viable, EMS-induced *Pu* mutations are cytologically normal. The head-specific GTP CH activity in each homozygote is very low, only 5 to 10% of normal. Like the spontaneous mutant strains, Pu^{r1} and Pu^{r331}, the EMS-induced mutants have adult body and prepupal activities that approach wild-type levels. Thus, we observe three phenotypically distinct classes of *Pu* mutants. One class has a dominant eye color and recessive lethal phenotype and alters GTP CH activity in prepupae and in adult head and body segments, although not necessarily to the same extent. A second class has a recessive lethal and eye color phenotype and affects prepupae and adult heads and bodies equivalently. A third class is homozygous viable, is recessive for eye color and shows a severe reduction in head-specific GTP CH not apparent in adult bodies or prepupae.

Effects of Punch mutations on GTP CH stability: The GTP CH activity of each segment or developmental stage was determined in crude extracts prepared from 30 individuals. The preparations were not adjusted so that extracts from different tissues had the same protein concentrations. The assay conditions were altered instead for optimal activity in each segment or stage. Therefore, a reasonable explanation for the head-specific reduction of GTP CH in some *Pu* mutants is that the mutations have rendered GTP CH unstable, and that the loss of activity is most severe in head extracts that have a much lower total protein concentration than extracts from adult body or prepupal tissues. We have attempted to address this possibility in several ways. We have added bovine serum albumin to the head homogenization buffers in order to increase the protein concentration to the level of the adult body extracts. The mutants showing head-specific loss of activity were assayed in this fashion, and in each case their head-specific GTP CH activities were identical to those obtained from the standard assay method (data not shown). Because the GTP CH enzyme appears to be rather hydrophobic (EDWARD WEISBERG, unpublished data), we have also tested Pu^{r1} and Pu^{rX17} extracts for GTP CH activity in the presence of low concentrations of nonionic detergents. This treatment, also, is ineffective in stabilizing the activity. Because our standard assay temperature of 42° is well above physiological temperatures, we considered the possibility

that the assay temperature itself was adversely affecting the activity. Therefore, extracts of heads and prepupae were assayed at 30° and 42° by taking time points at 5-min intervals for 20 min. *Pu* mutants assayed in this way were Pu^{r1}, Pu^{2}, Pu^{rX17}, Pu^{rAA4}, Pu^{rZ8}, Pu^{r331}, Pu^{rP1}, and Pu^{rP43}. In addition, we performed similar experiments for reduced activity mutants, *red*, ras^{2}, *pn*, and rs^{2}. No mutant gave clear-cut evidence for the production of an unstable GTP CH protein by this approach either (data not shown). Finally, we incubated extracts of pupae having high GTP CH activity levels at 53° for varying lengths of time prior to assay in order to determine whether a differential stability might be observed in those mutants with low head, high pupal activities. Decay of GTP CH activity was, in each case, indistinguishable from that of the parental, wild-type strains. Thus, for none of the mutants demonstrating head-specific GTP CH effects is there yet any evidence that the protein itself is defective.

The question arises whether *any Pu* mutation shows evidence of a physically altered GTP CH protein. In order to pursue this issue we turned to one of our EMS-induced *Pu* mutations, Pu^{rP43}. Pu^{rP43} is an unusual mutant in that it is a recessive lethal even though the Pu^{rP43}/wild-type heterozygote has 80–100% of wild-type GTP CH activity levels in all tissues. We have observed that prepupal and adult body activities in many mutants decrease to as little as 20% of normal without loss of viability. The inference based on our gene dosage experiments is that Pu^{rP43} should be contributing 30–50% of the total activity in the heterozygote, sufficient to survive as a homozygote. A second, lethal mutation elsewhere on the second chromosome might explain the results. However, extensive recombination efforts have failed to produce a homozygous viable individual, and the heterozygous combination of Pu^{rP43} with the very small deficiency, $Df(2R)Pu^{rF36}$, is similarly inviable. If a second mutation in a gene other than *Pu* is responsible for the lethal phenotype, it must be very close to the *Pu* locus. We have recently succeeded in purifying GTP CH from Drosophila heads and have determined that the enzyme appears to be a multimeric protein (E. WEISBERG and J. O'DONNELL, unpublished results). Thus, the characteristics of the Pu^{rP43} mutant could be explained as a positive complementation of mutant polypeptide subunits by normal subunits. If positive complementation of the mutant polypeptide is the correct explanation for the observation, then the putative hybrid protein of Pu^{rP43}/Pu^{+} might prove to be less stable than the wild-type enzyme. Accordingly, we incubated Pu^{rP43}/*wild type* and wild-type pupal extracts at 53° for varying lengths of time and assayed remaining activity. We chose to perform this experiment initially using pupal extracts because the pupal activity of Pu^{rP43}/Pu^{+} is consistently similar to wild-type activity indicating that mutant-wild type subunit combinations are apparently preserved through the extraction and assay procedures. Figure 2 illustrates the results of three such experiments in which extracts were heated for up to 5 min at 53°.

Pu^{rP43}/Pu^{+} extracts exhibited a much more rapid loss of activity in the 1- to 3-min heating interval than did Pu^{+}/Pu^{+} extracts. After this period, loss of activity proceeded at approximately the same pace in both extracts at least up to 20 min of heating in which Canton S and *dp cn a px sp* extracts retained

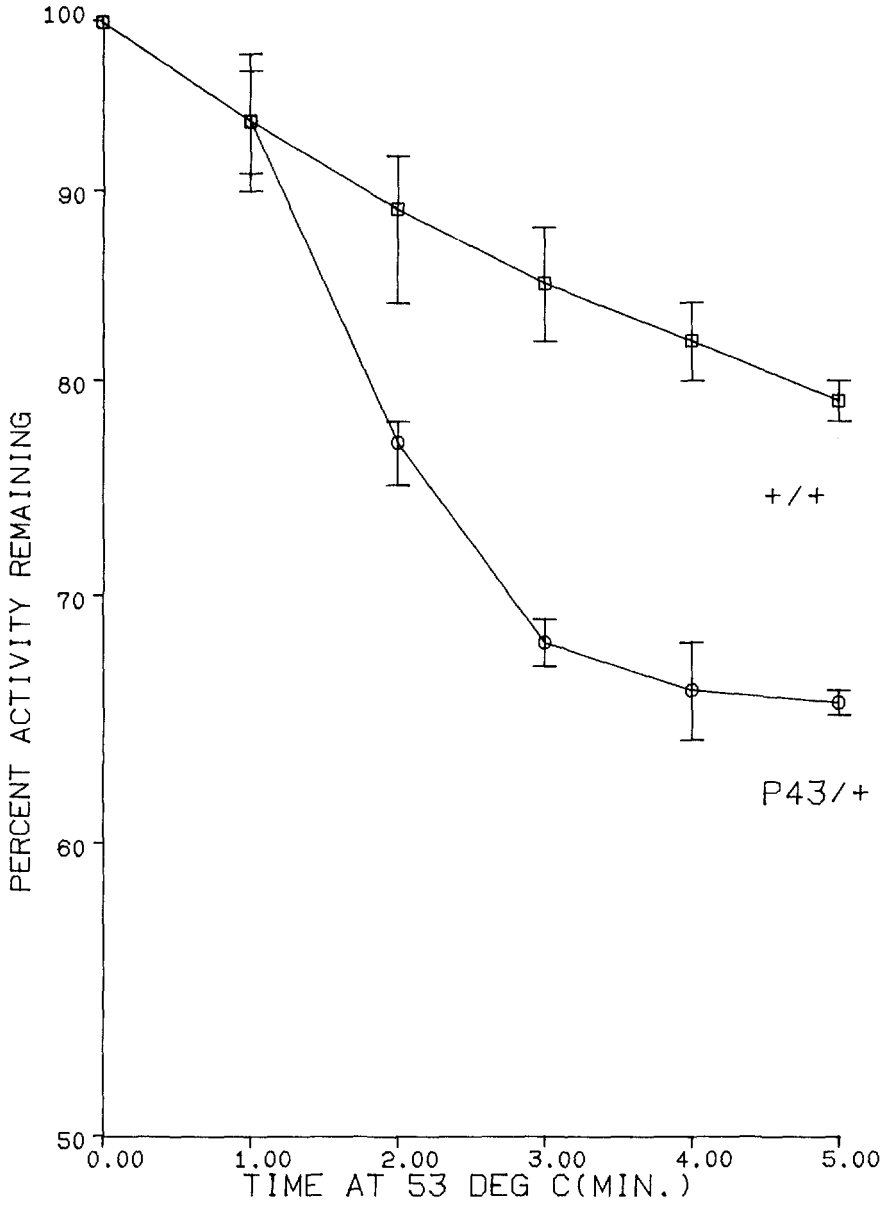

FIGURE 2.—GTP CH activity remaining after incubation of prepupal extracts at 53°. The Pu^+/Pu^+ strain was Canton S. The graph summarizes the average of three separate experiments. The bars indicate the range of relative activities obtained at each time point.

60% of their initial activity and Pu^{rP43}/Pu^+ retained about 40%. The results are consistent with a hypothesis that mutations in *Pu* alter GTP CH enzyme directly and that Pu^{rP43} produces a mutant polypeptide that is strongly complemented by normal GTP CH polypeptides.

DISCUSSION

Our survey of pteridine-defective mutants has revealed several strains with less than normal GTP CH activity in young adults. With the exception of Punch mutations, all have normal or nearly normal activity when heterozygous with wild-type alleles and have diminished activity only in the heads of homozygotes. EVANS and HOWELLS (1978) demonstrated, with the use of *X* chromosome aneuploid strains, that changes in the gene dosage of the *X*-linked loci, *pn* and *ras*, had no effect on GTP CH activity levels. We also find that the *Df(3R)red*^{31} heterozygote has normal levels of GTP CH (W. J. MACKAY and J. M. O'DONNELL, unpublished results). At least some alleles of each of the four loci, *pn*, *ras*, *rs*, and *red*, demonstrate very high levels of GTP CH activity in prepupae. We know nothing, at the moment, about the level of GTP CH protein in either the head or prepupal tissues of these strains, and we do not know whether the high specific activity of GTP CH in the prepupae of most mutant alleles is due to the same aberration that causes reduction in head-specific activity. We think it unlikely, however, that these loci are structural genes whose products are part of active GTP CH protein. Neither EVANS and HOWELLS (1978) in their investigation of the *pn* and *ras* loci nor we in studies of all of these loci (W. J. MACKAY and J. M. O'DONNELL unpublished data) have found any evidence that the mutants exert their negative effects through the action of enzymatic inhibitors. The tissue preparations are designed specifically to reduce the chances of such inhibition, and mixing of normal and mutant extracts offers no suggestion of such inhibitory action. Rather, the reduction in GTP CH activity in the heads of these mutants suggests a role for their normal products in the expression of the GTP CH structural locus or of the product of the locus. None of the primary physiological effects of any of the mutations are known at this time. The location of the *ras* locus in or near a purine auxotrophy region on the *X* chromosome has led NASH and HENDERSON (1982) to consider the possibility of a strong interdependence of purine and pteridine metabolism. There is, at the moment, no strong genetic or biochemical evidence speaking to this issue. Nevertheless we, too, have given some consideration to the notion that some or all of these loci could conceivably have primary roles within the purine pathways. Another possibility is that the loci define a pathway leading to a cofactor required by GTP CH in some, but not all, tissues. Our analysis of these loci has been hindered until recently by lack of anti-GTP CH antibody which will be important in defining the effects of the mutations on the enzyme. Studies using anti-GTP CH antibody and mutagenesis of each locus in an isogenic strain will be the most informative way to approach the functions of these loci.

It is of some interest that some eye color mutants revealed a higher than normal GTP CH activity in our initial survey experiments. Most notable among these are white and zeste. Although we have not yet pursued the source of the elevated activities, we note that they may be of value in understanding regulation and interactions in the pteridine pathway, and they will be the subject of future investigations.

Pu is the only locus examined so far that shows gene dosage effects of CH activity. Normally such observations in Drosophila are taken as a strong indication that the locus in question is a structural gene for a product of interest. However, acquiring corroborative evidence that the *Pu* locus encodes a GTP CH polypeptide has been difficult. The enzyme has proven rather recalcitrant to purification, and we have not, until recently, obtained an enzyme preparation sufficiently pure to use for immunization purposes. Furthermore, we have never been able to obtain active enzymes after polyacrylamide, starch, or agarose gel electrophoresis. Therefore, we have not been able to identify polypeptide alterations causing mobility changes. The heat stability characteristics of Pu^{rP43} provide a first indication that mutations in the Punch locus can, in fact, result in a physical change in the GTP CH protein. These data, in combination with the data showing a strict correlation between the number of Pu^{+} genes in a genome and GTP CH activity as well as the dosage response of most *Pu* mutations, are a strong indication that the Punch locus encodes GTP CH polypeptide. The spontaneous mutation, Pu^{r331}, is the only other *Pu* allele that fails to show clear-cut dosage effects. We have been unable to obtain evidence for protein instability in this mutant, and the characteristics of Pu^{r331} are, so far, unexplained.

The heat instability of Pu^{rP43}/Pu^{+} GTP CH is evident only for a fraction of the activity, whereas the remaining activity behaves like wild-type enzyme with respect to stability. We infer that the relatively unstable *vs.* relatively stable components of the curve reflect random associations of subunits with the unstable component representing a mostly Pu^{rP43} polypeptide composition. We expect to acquire further information with respect to this issue as our study of the protein proceeds. The heat inactivation experiments were performed using prepupal extracts. We performed similar experiments using adult head extracts to determine whether or not Pu^{rP43}/Pu^{+} head-specific activity was also unstable. In this case, Pu^{rP43}/Pu^{+} and Canton S extracts behaved identically with respect to the rate of activity loss on heating. In prepupal extracts the unstable component of the mutant amounts to no more than about 20–30% of the total activity. When we assay Pu^{rP43}/Pu^{+} adult heads we observe a 20% decrease in activity in the absence of any heating at all (see Table 7). It may be that the unstable component observed in the prepupal extracts cannot be seen in head extracts because it simply does not survive our standard extraction and assay procedures. Certainly some element of the head-specific enzyme seems far more sensitive to perturbation by mutation than the adult body and prepupal enzyme as may be seen by the characteristics of Pu^{r1}, Pu^{rAA4}, Pu^{rZ8} and Pu^{rX17}. The Pu^{rP43} enzyme behavior may be another reflection of this aspect of GTP CH expression. Alternatively, the subcellular environments of the enzyme in the adult head, where the enzyme is primarily localized in the eye, and in the prepupal stage, where the fat body expresses a major portion of the activity (W. J. MACKAY and J. M. O'DONNELL, unpublished observations) may be so different that crude extracts do not often permit meaningful physical comparisons. Recent evidence indicates, however, that EMS-induced mutations can alter the physical characteristics of head-specific GTP CH. The head-

specific activity of Pu^{rAA4} displays a severe cold sensitivity in the presence of 5% ethylene glycol, an observation that suggests an alteration in the hydrophobic bond interaction of the mutant protein (W. J. MACKAY, E. P. WEISBERG and J. M. O'DONNELL, unpublished data). Whether or not *Pu* is, in fact, the structural gene for GTP CH, the important point is that we can utilize its action on eye color, viability, and GTP CH activity as indicators of *Pu* mutant effects. With the appropriate use of these criteria and existing *Pu* mutations, we can manipulate the system to detect an unusually broad range of mutational effects, including those that have only very slight effects on GTP CH activity and those that result in tissue or stage-specific changes in expression. Studies of developmental regulatory mechanisms are often hindered by the fact that the range of detectable genetic changes are very limited, and only the most general and severe effects are apparent phenotypically. We might expect many regulatory effects to be more subtle. Therefore, the *Pu* locus should be a most informative system in which to pursue both *in vivo* and *in vitro* mutagenesis and studies of control mechanisms.

The mutation effects in the *Pu* locus reveal a complex pattern of gene expression. Whether the locus itself is structurally complex in the classical sense of the word (JUDD 1976) remains to be seen. Our mutagenesis frequencies indicate a significant frequency (1/2700) but not one that is inordinately high, especially considering the fact that we detect a broader than usual range of mutations. We have considered the possibility that the homozygous viable Punch mutations represent loss of function in a GTP CH gene whose major function is the generation of eye-specific pteridines and that a second GTP CH gene functioning primarily in other tissues remains unaffected. We do not think this is the case. Most mutations result in generalized and equivalent effects on GTP CH expression in all tissues and stages, consistent with a simple genetic organization. Furthermore, the EMS-induced *Pu* mutation, Pu^{rP43}, which was detected originally by its mutant eye color when trans to Pu^{r1}, shows evidence of expressing structurally altered GTP CH in the prepupal stage as well. We infer that at least some portion of the GTP CH polypeptide is expressed in both the adult and prepupal stages of the life cycle. The homozygous viable *Pu* mutations all demonstrate severe head-specific defects in GTP CH not observed in other tissues or stages. We do not yet know whether the effects of the mutations are exerted at the transcriptional, translational or posttranslational level. These tissue and stage-specific mutations will provide valuable source material for investigating the regulation of GTP CH expression in Drosophila development.

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