# TISSUE-SPECIFIC AND COMPLEX COMPLEMENTATION PATTERNS IN THE PUNCH LOCUS OF *DROSOPHILA MELANOGASTER*

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ABSTRACT

Mutations in the Punch locus result in loss of GTP cyclohydrolase activity, but all mutations do not affect the enzyme in the same way. There are at least three classes of Punch mutations. One class results in a dominant eye color, recessive lethal phenotype. A second class of mutations also causes a recessive lethal phenotype, but heterozygous mutants have normal eye color. They show loss of GTP cyclohydrolase function in all tissues where activity can be measured. Alleles comprising a third class are recessive eye color mutations that are homozygous viable. Individuals with this third type of mutation show loss of enzyme activity in the eye, but show normal or near-normal activity elsewhere. In order to examine the organization and function of this locus further, we have performed interallelic complementation tests on 25 Punch mutations, monitoring viability and enzyme activity in prepupae and adults. Most allele combinations are lethal. Those that complement do so in ways that are tissue- or stage-specific and unpredictable. Tests of mutants with tissue-specific phenotypes and of individuals mutant for complementing Punch lethal alleles lead us to conclude that Punch is a complex locus, both with respect to its organization and to its products.

THE Punch (*Pu*) locus of *Drosophila melanogaster* encodes a polypeptide of the enzyme guanosine triphosphate cyclohydrolase (GTP CH) (MACKAY and O'DONNELL 1983; MACKAY 1985; J. PAULUS, E. WEISBERG and J. M. O'DONNELL, unpublished results). This enzyme catalyzes the first reaction in the biosynthesis of pteridines, the conversion of guanosine triphosphate to dihydroneopterin triphosphate (BROWN 1971; FAN and BROWN 1976; YIM and BROWN 1976). Pteridines have a wide array of biological functions in all organisms (ZIEGLER and HARMSEN 1969; PHILLIPS and FORREST 1980). Derivatives of the pterin biopterin serve as cofactors for molybdenum hydroxylase enzymes such as xanthine dehydrogenase and aldehyde oxidase (WAHL *et al.* 1982; JOHNSON *et al.* 1984). Biopterin is also a cofactor for the aromatic amino acid hydroxylases, the responsibilities of which include the synthesis of several

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neurotransmitters (DANKA 1978; SCRIVER and CLOW 1980; LEVINE, MILLER and LOVENBERG 1981). In insects, including Drosophila, pterins also function as screening pigments in the eye (ZIEGLER and HARMSEN 1969; PHILLIPS and FORREST 1980). We have been investigating the role played by GTP CH in accommodating the diverse demands for pteridines throughout the life cycle of Drosophila. A central feature of our study has been a genetic analysis of the *Pu* locus.

In many respects, *Pu* appears to be a reasonably straightforward enzyme structural gene. GTP CH activity is most readily detected in the eyes of newly eclosed adults and in the fat body of late third-instar larvae and adults (FAN *et al.* 1976; EVANS and HOWELLS 1978; J. M. O'DONNELL and W. J. MACKAY, unpublished observations). Wherever enzyme activity can be assayed, it is strictly dependent on the number of $Pu^+$ genes present in the genome (MACKAY and O'DONNELL 1983; MACKAY 1985). An antigen recognized by antibodies generated against purified GTP CH varies linearly with $Pu^+$ dosage (WEISBERG and O'DONNELL 1985). Furthermore, the enzyme activity in extracts of several *Pu* mutant strains is heat labile, and the enzyme in at least one mutant strain has an altered isoelectric point (MACKAY and O'DONNELL 1983 and this report; E. WEISBERG, unpublished observations). On the other hand, *Pu* mutants display an array of phenotypes that suggests some complexity in the organization of the *Pu* locus. Some *Pu* mutations, including those first detected, cause dominant eye color and recessive lethal phenotypes (LINDSLEY and GRELL 1968; MACKAY and O'DONNELL 1983). Such mutants express <50% of normal enzyme activity in adult heads. The majority of *Pu* alleles, however, are recessive for eye color defects as well as lethality (MACKAY and O'DONNELL 1983; MACKAY 1985). This latter class includes *Pu* deficiencies. Deletion heterozygotes express 50% of wild-type levels of enzyme activity in all tissues where GTP CH can be detected. Individuals heterozygous for other alleles of this mutant class usually display activities ranging from 50–85%. The phenotypes resulting from a third class of *Pu* mutations differ in several respects from those of the first two classes. These mutations are homozygous viable, even though homozygotes exhibit severe reductions in eye pigmentation that should be indicative of complete or nearly complete loss of GTP CH function. These homozygous viable alleles, all of which are recessive, share an unusual feature. In each mutant strain, GTP CH activity is indeed severely defective in the adult eye, but all other tissues express the enzyme at normal or near-normal levels. We do not yet understand either the basis for the dominant eye color phenotype of the first class of *Pu* mutations or the tissue specificity of the third class. The experiments described in this report are intended to further define the levels and limits of complexity within this locus and to provide a genetic basis for understanding the functional organization of the *Pu* region.

## MATERIALS AND METHODS

*Strains*: All strains were maintained in half-pint milk 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 3 days, if strains were viable as homozygotes. For balanced lethal stocks, the number of parents was doubled.

Most of the strains used in these experiments were generated with ethyl methanesulfonate, as described previously (MACKAY and O'DONNELL 1983). Exceptions were *Df(2R)F36*, which was obtained by γ-irradiation, and *In(2R)Pu*$^{r1}$, *Pu*$^{r331}$ and *Pu*$^{2}$, which were spontaneous in origin. All mutant second chromosomes, except for *Pu*$^{r331}$ and *Pu*$^{2}$, also carry the recessive markers *dp*, *cn*, *a*, *px* and *sp*. The *Pu*$^{2}$ chromosome is marked with *a*, *px* and *sp*, whereas *Pu*$^{r331}$ carries a spontaneous allele of *cn* only. All homozygous lethal mutations were maintained in heterozygotes with the balancer chromosome *SM1*. Except where noted, all other mutations are described in LINDSLEY and GRELL (1968).

*Enzyme assays*: GTP cyclohydrolase assays were performed as previously described (MACKAY and O'DONNELL 1983), with the following modifications: 90 heads or 105 white pupae were homogenized in 600 μl and 700 μl, respectively, of the appropriate buffers. Extracts were incubated for 10, 15 and 20 min at 42°, or for 10, 20 and 30 min at 30°. Enzyme activities for the parental strain of most of the *Pu* mutants discussed here, *dp cn a px sp* (*Pu*$^{+}$), are reported as nanomoles of formate released per hour per milligram of protein. Enzyme activities for the *Pu* mutants are reported as a percentage of *Pu*$^{+}$ activity. The margin of error in these assays was ±5%.

For the heat-inactivation experiments, head and prepupal extracts were preheated at 53° for intervals of up to 20 min, followed by chilling in an ice-water bath. Samples then were assayed as described previously. Head and prepupal extracts of *Pu*$^{+}$ strains retain approximately 60% GTP CH activity after a 20-min incubation at 53°.

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

*Complementation analysis*: Reciprocal crosses of all pairwise combinations of 25 *Pu* mutants were carried out at 25°. In general, the genotypes of the *Pu*$^{lethal}$ strains are *dp cn Pu a px sp/SM1, al*$^{2}$ *Cy cn*$^{2}$ *sp*$^{2}$. Since the *Sm1* chromosome is also homozygous lethal, the normal viability of homozygous or heteroallelic *Pu* progeny should be one-third of the total progeny. The *dp cn Pu*$^{+}$ *a px sp* parental strain survives at the expected Mendelian frequency in control crosses. The viability of *Pu* combinations was normalized, therefore, to this frequency except in crosses involving homozygous *Pu* alleles, when the *Pu* heteroallelic combination should represent 50% of the total progeny. For most crosses, 800–1000 progeny were scored.

*Analysis of pteridine pigments*: Pterins were extracted by a modification of the procedure described by FAN *et al.* (1976). Twenty adults, aged less than 4 hr posteclosion, were weighed. Individuals were decapitated with a microscalpel, and heads were homogenized in a mixture of 0.5 ml of 29% ammonium hydroxide and 0.5 ml of chloroform. After a 1-min centrifugation in a microfuge, the aqueous layer was removed, heated for 1 min in a boiling water bath and briefly recentrifuged. Fluorescence levels of the resulting extracts were determined using a Perkin-Elmer Model 110 spectrofluorimeter with an excitation wavelength of 396 nm and an emission wavelength of 480 nm. Fluorescence levels of the mutants are reported as percentages of wild-type levels, with margins of error generally in the range of 10%.

*Determination of lethal phase*: Flies, mated and maintained at 25° or 16° for 3 days, were allowed to lay on yeasted agar plates. Embryos, 0–12 hr old, were placed in grids on fresh agar plates and were examined after a 24-hr incubation. Unhatched embryos were counted and, in some cases, larvae were examined at appropriate intervals after hatching.

## RESULTS

*Viability interactions between* Pu *alleles*: As a first step in the further characterization of mutations in the *Pu* locus, we determined the viability frequencies of heteroallelic combinations of five homozygous viable and 20 homozygous lethal *Pu* mutations. The GTP CH activities of all the viable strains and many of the lethal strains were described in a previous report (MACKAY and O'DONNELL 1983). We have provided a summary of those results in Table 1 as a guide in the following discussion. We first asked whether viability was com-

TABLE 1

*GTP cychlohydrolase activy in* Pu *mutants*

| Alleles | Prepupae | Adult body | Adult head |
|---|---|---|---|
| $Pu^{+}/Pu^{+}$ | 100 | 100 | 100 |
| Df(2R) F36, $Pu^{-}/Pu^{+}$ | 52 | 52 | 47 |
| $Pu^{2}/Pu^{+}$ | 19 | 34 | 32 |
| $Pu^{rI9}/Pu^{+}$ | 67 | 30 | 64 |
| $Pu^{rP1}/Pu^{+}$ | 48 | 28 | 53 |
| $Pu^{rP11}/Pu^{+}$ | 75 | 61 | 61 |
| $Pu^{rP21}/Pu^{+}$ | 52 | 48 | 63 |
| $Pu^{rP30}/Pu^{+}$ | 46 | 45 | 58 |
| $Pu^{rP42}/Pu^{+}$ | 84 | 63 | 68 |
| $Pu^{rP43}/Pu^{+}$ | 97 | 80 | 82 |
| $Pu^{rS47}/Pu^{+}$ | 52 | 55 | 67 |
| $Pu^{rZ19}/Pu^{+}$ | NT | NT | 87 |
| $Pu^{r1}/Pu^{r1}$ | 61 | 91 | 5 |
| $Pu^{rX17}/Pu^{rX17}$ | 94 | 80 | 6 |
| $Pu^{rZ8}/Pu^{rAA4}$ | 83 | 72 | 5 |
| $Pu^{rAA4}/Pu^{rAA4}$ | 77 | 41 | 8 |
| $Pu^{r331}/Pu^{r331}$ | 75 | 92 | 25 |

All enzyme activities are presented as percentages of activities in the parental strain, dp cn $Pu^{+}$ a px sp. The prepupal activity of the parental strain was 4.16 ± 0.06 nmol of formate per hour per milligram of protein in crude extracts (an average of three determinations). The adult body activity was 0.69 ± 0.12 nmol of formate per hour per milligram of protein in crude extracts (an average of six determinations). The adult head activity was 39.44 ± 2.15 nmol of formate per hour per milligram of protein in crude extracts. NT = not tested.

pletely normal in the homozygous viable mutant strains. This experiment was performed by selfing $Pu^{viable}/SM1$ heterozygotes and determining the survival frequency of homozygous $Pu^{viable}$ progeny relative to that of their heterozygous siblings. Four of the homozygous viable *Pu* alleles (*r1, rZ8, rX17, r331*) survive at 100% of expected, while the fifth, $Pu^{rAA4}$, survives at the somewhat reduced level of 62% of expected (Table 2).

Since mutants that are hemizygous for the homozygous viable alleles also survive, we anticipated the viability of these mutations in most *Pu* heteroallelic combinations. The results of experiments confirming our expectations are summarized in Table 2. Each homozygous viable *Pu* allele, in combination with any other viable or lethal *Pu* alleles, produces viable heteroallelic progeny, generally at or near expected Mendelian frequencies. The viability of 17 of the 125 combinations tested, however, was <70% of the expected frequency. All but one of the combinations involved $Pu^{rAA4}$ or $Pu^{rZ8}$, and half of them involved the lethal alleles *rV14, rV15, rW10* and *rX25*. All survivors have mutant eye colors, which will be described in greater detail below.

Next, we examined the viability characteristics of $Pu^{lethal}$ heteroallelic combinations. Nineteen of the 20 strains used in this experiment carry EMS-induced *Pu* mutations that cause recessive lethality. These mutations also result in a mutant eye color phenotype in combination with alleles of the homozygous

TABLE 2

*Viability of heteroallelic* Pu *progeny*

| | Homozygous viable | | | | | Homozygous lethal | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ♂ | ♀1 | *AA4* | *X17* | *Z8* | *331* | *I9* | *Z19* | *S47* | *P42* | *P30* | *P43* | *P11* | *2*[a] | *AA1* | *AA17* | *Z2* | *Z22* | *X25* | *W10* | *V15* | *V14* | *V9* | *V3* | *P21* | *P1*[a] | *F36*[b] |
| *1* | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 52 | + | + |
| *AA4* | + | 62 | + | + | + | + | 30 | + | + | + | + | + | + | + | + | + | + | 40 | 52 | 52 | 48 | + | + | 12 | 40 | 22 |
| *X17* | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| *Z8* | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 57 | + | 51 | 60 | 54 | 66 | 66 | 63 | + | 60 | + |
| *331* | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| *I9* | + | + | + | + | + | | | | 24 | 24 | 9 | | | | | | | | | | | | | | | |
| *Z19* | + | 42 | + | + | + | | | | 1 | 54 | | | | | | | | | | | | | | | | |
| *S47* | + | + | + | + | + | | | | + | 52 | | | | | | | | | | | | | | | | |
| *P42* | + | + | + | + | + | 36 | | + | 1 | | + | + | | | | | | | | | | | | | | |
| *P30* | + | + | + | + | + | 24 | 54 | 51 | | | + | + | | | | | | | | | | | | | | |
| *P43* | + | + | + | + | + | 8 | | | + | + | | | | | | | | | | | | | | | | |
| *P11* | + | + | + | + | + | | | | + | 42 | | | | | | | | | | | | | | | | |
| *2*[a] | + | + | + | + | + | | | | | | | | | | | | | | | | | | | | | |
| *AA1* | + | + | + | + | + | | | | | | | | | | | | | | | | | | | | | |
| *AA17* | + | + | + | + | + | | | | | | | | | | | | | | | | | | | | | |
| *Z2* | + | + | + | 69 | + | | | | | | | | | | | | | | | | | | | | | |
| *Z22* | + | + | + | + | + | | | | | | | | | | | | | | | | | | | | | |
| *X25* | + | 38 | + | 54 | + | | | | | | | | | | | | | | | | | | | | | |
| *W10* | + | 58 | + | 69 | + | | | | | | | | | | | | | | | | | | | | | |
| *V15* | + | 58 | + | 54 | + | | | | | | | | | | | | | | | | | | | | | |
| *V14* | + | 64 | + | 57 | + | | | | | | | | | | | | | | | | | | | | | |
| *V9* | + | + | + | 69 | + | | | | | | | | | | | | | | | | | | | | | |
| *V3* | + | + | + | 66 | + | | | | | | | | | | | | | | | | | | | | | |
| *P21* | 56 | 46 | + | + | + | | | | | | | | | | | | | | | | | | | | | |
| *P1*[a] | + | 18 | + | 66 | + | | | | | | | | | | | | | | | | | | | | | |
| *F36*[b] | + | 36 | + | + | + | | | | | | | | | | | | | | | | | | | | | |

All combinations marked with a "+" survive at frequencies >70% of expected. Numbers indicate the percentage of expected viability for those combinations displaying partial lethality. All other combinations are completely lethal. Progeny were raised at 25°. Progeny sample sizes ranged from 500–1000 individuals for each cross.

[a] $Pu^2$ is a dominant *Pu* allele, and $Pu^{P1}$ is partially dominant. All other alleles are fully recessive.

[b] Df(2R)F36,$Pu^-$

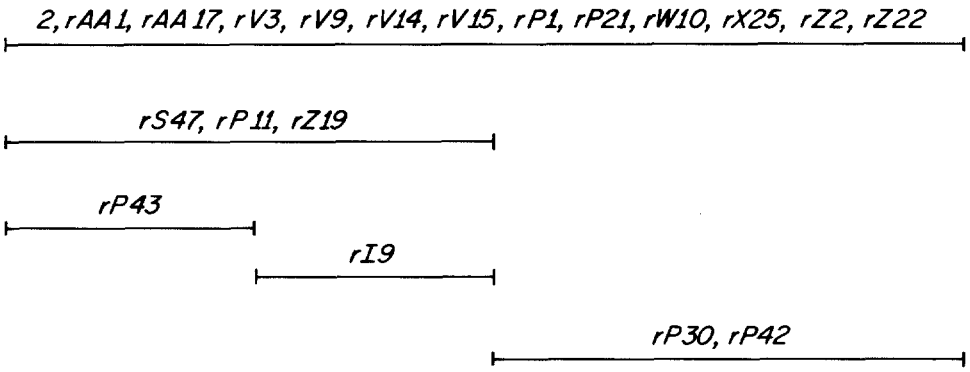


FIGURE 1.—Interallelic complementation for viability between *Pu* alleles. Overlapping bars indicate the absence of heteroallelic *Pu* progeny in the $F_1$ generation. Viability, indicated by non-overlapping bars, is defined as any reproducible survival of the *Pu* heteroallelic class, with no lower limit cutoff point.

viable class, although mutant/wild-type heterozygotes have normal eye color. The remaining strain has the dominant eye color mutation, $Pu^2$. Most heteroallelic combinations are also lethal. Seven of the mutant strains, however, exhibit interallelic complementation of the lethal phenotype (Table 2). They form five complementation groups, as shown in Figure 1. The frequency of survival varied in an allele-dependent fashion, from <1% to 100% of expected.

*Eye pigmentation in* Pu *heteroallelic individuals*: Four of the five homozygous viable *Pu* alleles cause severe eye color defects in homozygotes. This feature seems to dissociate *Pu* viability functions from *Pu* eye pigmentation functions. The survival of several of the heteroallelic *Pu* combinations allowed us to examine the relationship between *Pu* viability and eye color in more detail. Our studies utilized both quantitation of pteridines by fluorescence determinations and subjective visual comparisons, which we have found to be both sensitive and reliable. In order to improve visual resolution of pteridine levels, we eliminated ommochrome pigments from the eye by incorporating the mutation *cinnabar* (*cn*) in each genotype. Complete loss of pteridine and ommochrome pigments results in a white eye color.

1. $Pu^{viable}/Pu^{viable}$: The four homozygous *Pu* strains with severe eye color phenotypes are $Pu^{r1}$, $Pu^{rX17}$, $Pu^{rAA4}$ and $Pu^{rZ8}$. All $Pu^{viable}$ heteroallelic progeny derived from these strains similarly display extreme loss of pigmentation (data not shown). The eyes of the $Pu^{r331}$ homozygote have considerably higher levels of pigmentation. Heteroallelic combinations of $Pu^{r331}$ and the other $Pu^{viable}$ alleles produce progeny with an intermediate phenotype.

2. $Pu^{viable}/Pu^{lethal}$: We observe a wide range of eye pigmentation levels in the individuals from the 100 heteroallelic combinations tested; phenotypes range from white to cinnabar (essentially wild type for *Pu* function). Each combination can be placed in one of four groups, based on the amount of pigment produced. Individuals in group I have white eyes, and those in group II have pale orange eyes. Group III individuals have considerably more pigmentation, but are readily distinguishable from wild type throughout adult life.

TABLE 3

*Eye pigmentation and GTP cyclohydrolase activity in* $Pu^{viable}/Pu^{viable}$ *and* $Pu^{lethal}/Pu^{viable}$ *strains*

| *Pu* genotype | Relative enzyme activity in heads[a] | Relative pteridine levels in heads[b] | Eye color class[c] |
|---|---|---|---|
| $Pu^+/Pu^+$ | 100 | 100 | V |
| *r1/r1* | 5 | 8 | I–II |
| *rX17/rX17* | 6 | 16 | II |
| *rZ8/rZ8* | 5 | 12 | II |
| *rAA4/rAA4* | 8 | 25 | II |
| *r331/r331* | 25 | 36 | II<br>III–IV |
| *2/r1* | 2 | 3 | I |
| *rI9/r1* | 3 | 4 | I |
| *rP30/r1* | 2 | 3 | I |
| *rZ19/r1* | 3 | 4 | I |
| *rP11/r1* | 9 | 12 | II |
| *rP43/r1* | 17 | 15 | II |
| *rP42/r1* | NT | 24 | II–III |
| *rS47/r1* | 9 | 13 | II |
| *rP21/r1* | 1 | 2 | I |
| *rZ2/r1* | NT | 2 | I |

[a] The enzyme activity of the parental strain was 39.44 2.15 nmol of formate per hour per milligram of protein in crude extracts (three determinations). NT = not tested.

[b] Pteridine levels are reported as relative fluorescence units per wet weight of adult animals, normalized to wild type (two–four determinations).

[c] Classification as described in text.

Group IV individuals can only be distinguished from wild type in very young adults. Group V is the wild-type class.

All 16 $Pu^{viable}/Pu^{lethal}$ combinations that survive at reduced frequencies can be placed in groups I or II. The remaining heteroallelic classes, which survive normally, may fall in any one of the four pigmentation groups. Some examples of the results are presented in Table 3.

3. $Pu^{lethal}/Pu^{lethal}$: Surprisingly, the eyes of all $Pu^{lethal}/Pu^{lethal}$ heteroallelic survivors are quite highly pigmented, regardless of the viability characteristics of the combination. Adults arising from combinations that are completely viable and those that survive at <1% of expected frequencies have virtually indistinguishable eye phenotypes (Table 4). All have more pigment than is found in any of the $Pu^{viable}$ homozygotes except $Pu^{r331}$.

Some of the alleles in these combinations, such as $Pu^{rP42}$ and $Pu^{rP43}$, appear to be leaky, based on their enzyme activities and their phenotypes in $Pu^{viable}/Pu^{lethal}$ combinations (Tables 1 and 3). Other alleles appear to have more serious defects, but in heteroallelic combinations they provide clear evidence for complementation of eye color as well as viability. For instance, $Pu^{rZ19}/Pu^{rP30}$

TABLE 4

*Characteristics of* $Pu^{lethal}/Pu^{lethal}$ *heteroallelic strains*

| Strain | Relative viability | | | Relative GTP CH activity[a] | | Relative eye pigmentation | |
|---|---|---|---|---|---|---|---|
| | 16° | 25° | 29° | Adult head | Prepupae | Relative fluorescence[b] | Eye color class[c] |
| $Pu^{+}/Pu^{+d}$ | 100 | 100 | 100 | 100 | 100 | 100 | V |
| $Pu^{rP43}/Pu^{rP42}$ | 5 | 100 | 75 | 35 | 24 | 33 | IV |
| $Pu^{rP43}/Pu^{rP30}$ | 2 | 90 | 63 | 29 | 21 | 43 | III |
| $Pu^{rp43}/Pu^{r19}$ | 0 | 8 | 8 | NT | NT | 35 | III |
| $Pu^{rP11}/Pu^{rP42}$ | 5 | 84 | 69 | 50 | 29 | 42 | IV |
| $Pu^{r19}/Pu^{rP30}$ | 1 | 24 | 12 | 20 | 5 | 36 | III |
| $Pu^{rS47}/Pu^{rP42}$ | 22 | 98 | 84 | 34 | 26 | 42 | IV |
| $Pu^{rS47}/Pu^{rP30}$ | 24 | 52 | 70 | 25 | 25 | 30 | III |
| $Pu^{rZ19}/Pu^{rP42}$ | 0 | 1 | 2 | 28 | 18 | 30 | III |
| $Pu^{rZ19}/Pu^{rP30}$ | 0 | 54 | 42 | 20 | 6 | 33 | III |
| $Pu^{r19}/Pu^{rP42}$ | 0 | 30 | 41 | 27 | 13 | 25 | III |
| $Pu^{rP11}/Pu^{rP30}$ | 16 | 64 | 57 | 29 | 28 | 39 | III |

[a] Enzyme activity for the $Pu^{+}$ strain was 39.44 ± 2.15 nmol of formate released per hour per milligram of protein in crude head extracts and 4.16 ± 0.06 nmol of formate released per hour per milligram of protein in crude prepupal extracts (three determinations each). NT = not tested.

[b] Fluorescence is reported as units per wet weight of adult animals, normalized to wild type (three–four determinations).

[c] Classes are described in text.

[d] This strain, which has the genotype *dp cn a px sp*, was the parental strain from which all mutants in this table were derived.

and $Pu^{r19}/Pu^{rP30}$ have strongly pigmented eyes, while each of the alleles produces a nearly white-eyed individual when combined with any homozygous viable mutant alleles except $Pu^{r331}$.

*Rescue of lethality and mutant eye color with an extra dose of the* Pu *region*: The complementing *Pu* alleles were derived from a multiply marked strain that had been made isogenic before mutagenesis. It was possible, nevertheless, that lethal mutations elsewhere on the second chromosome shared some of the responsibility with the *Pu* lesions for the lethality of *Pu* heteroallelic mutants. Because the viability characteristics of the *Pu* mutants are an important part of this study, it was important to eliminate from consideration the possible role of secondary lethal mutations.

We first re-tested approximately 1000 parental second chromosomes for the presence of accumulated lethal mutations in the stock, and we found none. We then asked whether an extra dose of $Pu^{+}$ could rescue nearly lethal heteroallelic combinations. For this experiment we used an insertional translocation of the *Pu* region, *T(Y;2)JL-11, 56F–57F* (LYTTLE 1984). This translocated $Pu^{+}$ locus is normal with respect to GTP CH activity (MACKAY and O'DONNELL 1983). The results of the experiment are presented in Table 5.

The analysis is slightly complicated by a reduction in the viability frequency of male aneuploids. The impaired survival of such individuals appears to be caused by the extra dose of the region, as opposed to the effects of the physical

TABLE 5

*Rescue of lethality in* Pu *heteroalleles*

| | Genotype[a] | No. of progeny |
|---|---|---|
| Cross 1 ($Pu^+/Pu^+$) | $Pu^+$; Df(2R)56F-57F/$Pu^+$ ♂ | 223 |
| | $Pu^+$;$Pu^+$/$Pu^+$ ♂ | 65 |
| | $Pu^+$/$Pu^+$ ♀ | 223 |
| | $Pu^+$/Df(2R)56F-57F ♀ | 0 |
| Cross 2 ($Pu^{rP43}/Pu^{rI9}$) | $Pu^+$; Df(2R)56F-57F/$Pu^{I9}$ ♂ | 141 |
| | $Pu^+$; Df(2R)56F-57F/$Pu^+$ ♂ | 117 |
| | $Pu^+$; $Pu^{rP43}$/$Pu^+$ ♂ | 88 |
| | $Pu^+$; $Pu^{rP43}$/$Pu^{rI9}$ ♂ | 61 |
| | $Pu^{rP43}$/$Pu^{rI9}$ ♀ | 16 |
| | $Pu^{rP43}$/$Pu^+$ ♀ | 133 |
| | $Pu^{rI9}$/Df(2R)56F-57F ♀ | 0 |
| | $Pu^+$/Df(2R)56F-57F ♀ | 0 |
| Cross 3 ($Pu^{rZ19}/Pu^{rP42}$) | $Pu^+$; Df(2R)56F-57F/$Pu^{rP42}$ ♂ | 158 |
| | $Pu^+$; Df(2R)56F-57F/$Pu^+$ ♂ | 80 |
| | $Pu^+$; $Pu^{rZ19}$/$Pu^+$ ♂ | 93 |
| | $Pu^+$; $Pu^{rZ19}$/$Pu^{rP42}$ ♂ | 70 |
| | $Pu^{rZ19}$/$Pu^{rP42}$ ♀ | 15 |
| | $Pu^{rZ19}$/$Pu^+$ ♀ | 135 |
| | $Pu^{rP42}$/Df(2R)56F-57F ♀ | 0 |
| | $Pu^+$/Df(2R)56F-57F ♀ | 0 |

[a] Cross 1. T(Y;2)JL-11,56F-57F;Df(2R)56F-57F/cn $Pu^+$ bw ♂ × cn $Pu^+$ bw/cn $Pu^+$ bw ♀

Cross 2. T(Y;2)JL-11,56F-57F;dp cn $Pu^{rP43}$ px sp/Df(2R)56F-57F ♂ × dp cn $Pu^{rI9}$ a px sp/SM1, $al^2$ Cy $cn^2$ $Pu^+$ $sp^2$ ♀

Cross 3. T(Y;2)JL-11,56F-57F;dp cn $Pu^{rZ19}$ a px sp/Df(2R)56F-57F ♂ × dp cn $Pu^{rP42}$ a px sp/SM1, $al^2$ Cy $cn^2$ $Pu^+$ $sp^2$ ♀

disruption of the chromosomes (compare lines 1 and 2 of the control cross 1). Further experiments to define the source of this viability effect are in progress. In spite of this consideration, it is evident that the heteroallelic individuals survive at considerably higher frequencies when an additional $Pu^+$ locus is introduced (compare lines 4 and 5 in crosses 2 and 3). These individuals have a very slight eye color defect immediately after eclosion, but rapidly attain wild-type pigmentation levels. The eyes of heteroallelic euploid males are highly pigmented, but are permanently mutant in phenotype. Thus, the addition of an extra 56F–57F chromosomal region rescues both the viability and eye pigmentation defects associated with *Pu* mutations.

*GTP CH activity in heteroallelic individuals*: In a previous publication we reported a correlation between GTP CH activity in the heads of young $Pu^{r1}$/$Pu^{lethal}$ adults and the degree of eye pigmentation (MACKAY and O'DONNELL 1983). We assayed the $Pu^{viable}/Pu^{lethal}$, $Pu^{viable}/Pu^{viable}$ and $Pu^{lethal}Pu^{lethal}$ heteroallelic progeny generated in the *Pu* viability tests to ascertain whether or not pigmentation and enzyme activity were related in these additional cases. Every heteroallelic mutant shows this relationship (Tables 3 and 4). Typically, surviving progeny from $Pu^{lethal} \times Pu^{lethal}$ crosses have more pigmentation than most

TABLE 6

Pu *mutant lethal phase determination*

| Cross | Temperature | No. of Embryos | No. of unhatched | Percentage[a] | No. of dead larvae | Percentage[a] |
|---|---|---|---|---|---|---|
| *dp cn a pxsp* × *dp cn a pxsp* | 25° | 1052 | 144 | 13 | 16 | 2 |
| *dp cn a pxsp/SM1* × *dp cn a pxsp/SM1* | 25° | 600 | 66 | 11 | 162 | 27 |
| Canton S × Canton S | 25° | 500 | 21 | 4 | 12 | 2 |
| $Pu^{rP30}$/SM1 × $Pu^{rP30}$/SM1 | 25° | 422 | 151 | 36 | 42/160[b] | 26 |
| $Pu^{rZ19}$/SM1 × $Pu^{rZ19}$/SM1 | 25° | 510 | 153 | 30 | 61/300[b] | 20 |
| $Pu^{rP42}$/SM1 × $Pu^{rP42}$/SM1 | 25° | 510 | 171 | 34 | ND | — |
| $Pu^{rP43}$/SM1 × $Pu^{rP43}$/SM1 | 25° | 522 | 197 | 37 | ND | — |
| $Pu^{rP21}$/SM1 × $Pu^{rP21}$/SM1 | 25° | 502 | 191 | 38 | ND | — |
| $Pu^{rZ22}$/SM1 × $Pu^{rZ222}$/SM1 | 25° | 560 | 151 | 27 | ND | — |
| $Pu^{2}$/SM1 × $Pu^{2}$SM1 | 25° | 500 | 219 | 44 | ND | — |
| $Pu^{rAA4}$ × $Pu^{rAA4}$ | 25° | 544 | 146 | 27 | ND | — |
| $Pu^{rP30}$/SM1 × $Pu^{rZ19}$/SM1 | 25° | 500 | 114 | 23 | 96 | 20 |
| $Pu^{rP30}$/SM1 × $Pu^{rZ19}$/SM1 | 16° | 520 | 203 | 40 | 104 | 20 |

[a] Percentage of total embryos scored.
[b] For these crosses a subset of the embryos collected were followed through the larval stage. ND = not determined.

$Pu^{viable}/Pu^{lethal}$ individuals. They also have higher GTP CH activities, ranging from 20–50% of wild-type levels.

Next, we wished to consider the relationship between viability and GTP CH activity. Viability frequencies in most experiments were determined by scoring eclosed adults. The data presented in Table 6, however, demonstrate that virtually all *Pu* lethality occurs during embryogenesis. That the observed larval death is due to *Sm1/Sm1* lethality is established by crosses 1 and 2 in Table 6. The *SM1* lethal period occurs during the first larval instar. Little lethality is seen at subsequent larval stages or during pupation. The mutants providing the data for these experiments are representative of almost all *Pu* homozygous and heteroallelic strains. Since we cannot detect GTP CH activity in embryos, however, a direct comparison of embryonic viability frequencies and GTP CH activity has been impossible. We have asked, instead, whether enzyme activity at pupariation and in other adult tissues has any relationship to viability characteristics. As in the comparison of the degree of adult eye pigmentation and viability, no correlation can be made between viability and prepupal or adult enzyme activity (Table 4). Although lack of a correlation is particularly evident in the *viable/viable* and *viable/lethal* data where survival may be very good while enzyme activity in the head is very low, it is also seen in *lethal/lethal* survivors. There are examples in which viability at 25° is normal, even though enzyme activity is quite reduced. Interestingly, the inverse of these characteristics is also observed. For instance, $Pu^{rZ19}/Pu^{rP42}$ individuals have 28% of wild-type enzyme activity levels in adult heads, and $Pu^{rP43}/Pu^{rP30}$ progeny have 29%. Yet, the former survive at only 1% of the expected frequency, while the latter survive at an essentially normal frequency at 25°.

Our previous studies had shown that viability of *Pu* homozygotes was apparently related to or at least correlated with GTP CH activity in tissues and developmental stages other than adult heads (Table 1 and MACKAY and O'DONNELL 1983). All surviving *Pu* homozygotes have high levels of enzyme activity at pupariation and in adult bodies. The lethal mutations in *Pu*, on the other hand, generally reduce adult head, adult body and prepupal activities to a great extent. That correlation does not hold for all surviving *lethal/lethal* heteroallelic combinations. Individuals from all 11 viable *lethal/lethal* combinations have reduced prepupal activities ranging from 29% to only 5% of wild type. In 8 of the 11 combinations, the relative activities in prepupae are lower than those in adult heads. Again, though, there is no apparent correlation between viability and enzyme activity (Table 4). Note, for example, that $Pu^{rP43}/Pu^{rP42}$ survives at a normal frequency, whereas $Pu^{rZ19}/Pu^{rP42}$ is barely viable, even though these two heteroallelic strains have very similar prepupal GTP CH activities. $Pu^{rP11}/Pu^{rP30}$ survives at 64% of the expected viability and has 28% of wild-type activity levels in the prepupal stage. $Pu^{rZ19}/Pu^{rP30}$ survives almost as well (54% of expected), yet has only 6% of normal activity levels in prepupae.

*GTP CH stability in* $Pu^{lethal}/Pu^{lethal}$ *heteroallelic strains*: It is conceivable that the failure to observe clear correlations in all cases between viability and enzyme activity in prepupae and adult heads is due to instability of the mutant proteins in the assay conditions used. We attempted to address this issue in two ways. For these experiments, we took advantage of the fact that heteroallelic survivors of viability tests are fertile. We temporarily maintained *Pu* heteroallelic strains in order to provide adequate progeny numbers to perform the necessary tests (these strains were also the source of prepupae for assays described in the previous section). First, we performed a series of assays on head and prepupal extracts at 30°, rather than at the usual 42° assay temperature. If those strains demonstrating very low prepupal activities make heat-labile proteins, we might anticipate improved relative activities at the lower assay temperature. If, on the other hand, a particular mutant protein were cold-sensitive, we might observe adverse effects when the temperature is reduced. In no case, however, were prepupal or adult head activities relative to wild type different at 30 and 42° (data not shown).

We then turned to heat-lability tests of the mutants. A representative sample of the results is shown in Figure 2. Most of the 11 heteroallelic strains produce a GTP CH activity that is labile in head extracts, although not surprisingly, the degree of lability is allele combination specific. The most unstable activity is found in $Pu^{rZ19}/Pu^{rP42}$, a heteroallelic strain that survives at only 1% of the expected frequency. We observe no general trend, however, between enzyme lability and lethality.

Because several of the heteroallelic strains have lower relative activities in the prepupal stage than in adult heads, it might be anticipated that the prepupal activities in such strains are simply less stable. We observe just the opposite. Six of the ten strains produce a prepupal enzyme that is more stable than the head enzyme. Three of these, in fact, are completely normal in their

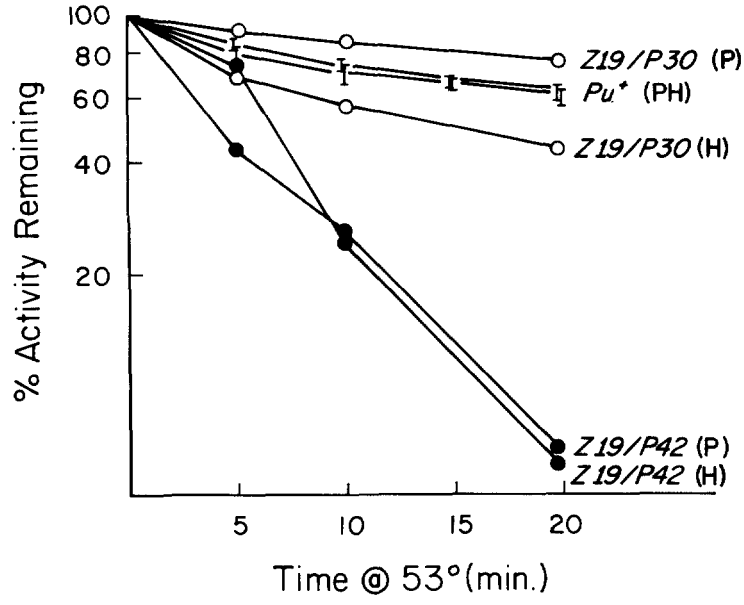


FIGURE 2.—Heat stability of GTP CH activity in head and prepupal extracts. The $Pu^{+}$ strain was *dp cn a px sp*. Bars indicate the range of relative wild-type activity obtained at each time point from five separate experiments.

rate of prepupal enzyme inactivation, including the two strains with the lowest prepupal activities, $Pu^{rI9}/Pu^{rP30}$ and $Pu^{rZ19}/Pu^{rP30}$.

We cannot absolutely rule out the possibility that assay conditions in some cases contribute to the lack of correlation between viability and enzyme activity. We can, however, find no general pattern of *in vitro* characteristics that could explain all cases. It seems there is some aspect of *Pu* function or component of the *Pu* product that is different in each developmental stage where function can be monitored. The following section describes a series of experiments that reinforce this conclusion.

*Effects of temperature on viability interactions between* Pu *alleles*: The combination of *in vivo* and *in vitro* studies just described has revealed what appears to be a partial independence of *Pu* expression with respect to developmental stage. Although considerable progress has been made in the physical characterization of the protein, it is complex in structure and behavior (WEISBERG and O'DONNELL, 1985). Until physical studies of the protein have reached a more complete state, conclusions reached partially on the basis of *in vitro* experiments must be regarded cautiously. It is important to define *in vivo* parameters of mutant behavior as extensively as possible. With these considerations in mind, we expanded our study of *Pu* lethal effects to include complementation analysis performed at 29° and 16°, using the lethal strains exhibiting interallelic complementation for viability at 25°. The results are summarized in Table 4. Although some of the heteroallelic combinations do not survive as well at 29° as at 25°, the reduction in viability is not dramatic in any case. All combinations, however, regardless of their viability at 25 or 29°,

TABLE 7

*Temperature effects on viability of* viable/viable *and* viable/lethal Pu *progeny*

| Cross | Relative viability[a] | |
|---|---|---|
| | 16° | 25° |
| dp cn a px sp/SM1 × dp cn a px sp/SM1 | 100 | 100 |
| $Pu^{rAA4}$/SM1 × $Pu^{rAA4}$/SM1 | 82 | 62 |
| $Pu^{rZ8}$/SM1 × $Pu^{rZ8}$ | 106 | 90 |
| $Pu^{rX17}$/SM1 × $Pu^{rX17}$ | 86 | 106 |
| $Pu^{rZ8}$/SM1 × $Pu^{rX17}$ | 104 | 102 |
| $Pu^{rP43}$/SM1 × $Pu^{r1}$ | 106 | 89 |
| $Pu^{rS47}$/SM1 × $Pu^{r1}$ | 110 | 85 |
| $Pu^{r19}$/SM1 × $Pu^{r1}$ | 86 | 99 |
| $Pu^{rZ19}$/SM1 × $Pu^{r1}$ | 102 | 75 |
| $Pu^{rP11}$/SM1 × $Pu^{r1}$ | 92 | 80 |
| $Pu^{rP30}$/SM1 × $Pu^{r1}$ | 92 | 72 |
| $Pu^{rP43}$/SM1 × $Pu^{r1}$ | 86 | 98 |

[a] Percentage of expected viability of the *Pu* homozygous or heteroallelic class of progeny.

are seriously affected by growth at 16°. Four strains are completely lethal at 16°, and the remaining seven survive at greatly reduced frequencies. Table 6 presents data establishing the lethal period of one of the heteroallelic strains. We have carefully examined all combinations and find no evidence of lethality at later periods. As in all other experiments, it is impossible to predict the degree of *in vivo* cold-sensitivity on the basis of any other measured parameter in any developmental stage.

Regardless of the severity of cold-sensitivity with respect to viability, the lower culture temperature has no apparent effect on eye pigmentation in the survivors. Eye pigmentation in individuals raised at 16, 25 and 29° is indistinguishable. Thus, $Pu^{lethal}/Pu^{lethal}$ heteroallelic strains are cold-sensitive for viability but not for eye pigmentation functions.

The substitution of a $Pu^{lethal}$ allele in the heteroallelic strains with a $Pu^{viable}$ allele in the heteroallelic strains relieves cold-sensitivity. We have tested several $Pu^{viable}$ homozygotes, as well as *viable/viable* and *viable/lethal* heteroallelic combinations for survival at 16°. Representative results are shown in Table 7. In no case were the viability frequencies significantly lower than those at 25°. In fact, many strains have somewhat higher viability when raised at the lower temperature. The eyes in all strains were indistinguishable from those of their counterparts raised at 25°.

## DISCUSSION

In this and a previous report (MACKAY and O'DONNELL 1983) we have described several classes of *Pu* mutations. There are two types of dominant mutations; both impart recessive lethal phenotypes. One, resulting from heterochromatic breakpoint rearrangements, produces a variegated eye color phenotype. The other dominant class causes a nonvariegated eye color phenotype,

and mutants appear cytologically normal. There are three classes of recessive eye color mutations. The first contains the majority of all *Pu* mutations; these are noncomplementing recessive lethals. Some recessive eye color mutations, however, are capable of interallelic complementation for viability. We place these in a separate class for the purposes of discussion, although we have no evidence that they differ from the former class either in distribution of mutant sites or in types of mutations. Finally, approximately 10% of all *Pu* mutations are homozygous viable, even though they cause eye color defects of a severe nature. We shall consider, primarily, the last two categories.

Mutants homozygous for the viable *Pu* alleles and those carrying complementing lethal alleles exhibit profound differences in their characteristics, even though in many cases their viability frequencies are comparable. With the exception of $Pu^{r331}$, which has a considerable amount of pigmentation in the adult eye and has other characteristics that distinguish it from other homozygotes (MACKAY and O'DONNELL 1983), the homozygous mutants show an extreme loss of enzyme activity and pigmentation in the adult eye. The homozygous viable mutations have shown no capacity for interallelic complementation of eye color, in combination with any other alleles. Eye pigment by these mutant allele combinations is strictly additive. In contrast, all surviving $Pu^{lethal}$ heteroallelic progeny, without exception, have significantly higher levels of eye pigmentation than those found in any of the $Pu^{viable}$ mutants except $Pu^{r331}$. In some cases, high levels of pigmentation occur in combinations of two quite leaky alleles. There are other lethal alleles, though, that contribute little or no GTP CH activity when combined with any viable or wild-type *Pu* allele but that demonstrate clear evidence of positive complementation of eye activity in lethal allele combinations.

In every case examined, regardless of the condition of the *Pu* locus or the origin of the strain, eye pigmentation and GTP CH activity in the adult head are closely correlated. This correlation seems only logical since the synthesis of all pterins absolutely depends on GTP CH activity (BROWN 1971). The point is not a trivial one, however, since the correlations begin to break down as we consider enzyme activities and mutant phenotypes in other adult tissues and developmental stages. We must be concerned that any *in vitro* activity measurement is a misrepresentation of the true state of the *Pu* product. The agreement between pigmentation and enzyme activity in the eye demonstrates that, in this case, the mutant effects we quantitate *in vitro* with the assay of GTP CH activity accurately reflect the condition of the locus and enzyme activity *in situ*. Initially, we tailored our assay conditions to each tissue or stage under examination, and we have attempted various means of stabilizing the enzyme *in vitro* and *in vivo*. No instance of mutant effects that seem independent or partially independent of those in other tissues or stages has been resolved by our efforts. The lack of correlation could be related to a requirement for a threshold level of enzyme activity for viability. The individuals that survive conceivably could be those capable of producing a higher level of functional product than their sibs and therefore are not truly representative of that mutant class. The fact that the eye function of surviving *lethal/lethal*

individuals is greater than anticipated in all cases could support this hypothesis. However, we do not observe similarly high levels of function in the prepupal stage. Therefore, we do not believe that the characteristics of the survivors are biasing our conclusions. We are convinced that the apparent independence of mutations within *Pu* and the rather complicated patterns of interallelic complementation are indications of complexities in the organization of the locus and of the product or products of that locus.

The eye-specific class of mutations remains the clearest example of the apparent complexity of the locus, but the complementing *Pu* lethal mutations suggest that the functional organization of *Pu* and its products may extend beyond an "eye" *vs.* "noneye" dichotomy of expression. Alleles complement in ways that are complex, tissue- or stage-specific, and completely unpredictable. The viability frequency, however, is the only characteristic observed to be completely normal in any heteroallelic combination. The independence of expression is, instead, one of degrees, which indicates that some common element of the locus is required wherever *Pu* function can be measured. Even in those combinations in which complementation for viability is complete, the *Pu* product is not entirely normal, as evidenced by the cold-sensitivity of the progeny. We have never observed even a slight indication that the normal product of *Pu* is in any way cold-sensitive, and an entire class of *Pu* alleles, the homozygous viable mutations, fails to show this phenotype.

That the complementing lethal *Pu* alleles affect the *Pu* product in ways quite different from the viable *Pu* alleles is further emphasized by the characteristics of the homozygous viable allele, $Pu^{rAA4}$. This mutation shows clear evidence of an embryonic lethal effect (Table 6). It is hypomorphic, as demonstrated by the decrease in viability when $Pu^{rAA4}$ is made heterozygous with a *Pu* deficiency. In spite of the fact that an embryonic function is quite obviously altered, the cold-sensitivity that seems to be a universal trait of the complementing lethal heteroalleles is not seen in $Pu^{rAA4}$.

The cold-sensitivity of complementing lethal heteroallelic individuals raises interesting questions with respect to the nature of the *Pu* product. Cold-sensitive mutant phenotypes have been attributed to an assortment of defects, including lability of protein products (PULLMAN *et al.* 1960; SHUKUYA and SCHWERT 1960), conformational changes in allosteric proteins (O'DONOVAN and INGRAHAM 1965), inability to assemble protein complexes (GUTHRIE, NASHIMOTO and NOMURA 1969) and inability to process RNA properly (DAVIS and WILLIAMS 1982). GTP CH isolated from adult Drosophila heads is quite hydrophobic compared with most soluble enzymes. It purifies as a very large complex (ca. 550,000 daltons) (WEISBERG and O'DONNELL, 1985). The characteristics of the protein had led us to anticipate the possibility of obtaining cold-sensitive *Pu* mutations. What we did not anticipate is that the cold-sensitivity is apparently restricted to the embryo. We looked for evidence of *in vitro* cold-sensitivity in GTP CH in later stages. We found none. However, the lowest temperature at which we assayed the enzyme is still well above that at which cold-sensitive lethality was observed. We would have preferred to perform the *in vitro* experiments at the phenocritical temperature. Unfortunately,

the time required at low assay temperatures to accumulate sufficient product for accurate quantitation would create a variety of artifacts. At the assay temperatures we used, we did not necessarily expect to see a dramatic effect, but we would have detected even slight differences. These experiments address only the possibility that the enzyme is cold-labile. Temperature shift experiments and enzyme assays of mutants raised at temperatures other than 25° are currently under way to examine further the possibility that the *Pu* product expressed after embryogenesis is also cold-sensitive but lacking an obvious phenotype.

Regardless of the results of the preceding experiments, it is clear that the eye function of the *Pu* locus is not cold-sensitive. We have never observed changes in the accumulation of eye pigment in mutants raised at 16°. Pigment accumulation is such a sensitive indicator of GTP CH activity that we can detect by eye color alone activity changes as small as 5–10%. The cold-sensitive phenotype is not exhibited in the eye and may be restricted to embryonic tissues.

The apparent restriction of the cold-sensitive behavior to the embryos of heteroallelic strains has led us to question whether the product of *Pu* that we are measuring by embryonic viability is, in fact, GTP CH. All results to date are consistent with the production of GTP CH by *Pu*. The data may not be inconsistent with other interpretations, however. If *Pu* were to encode a regulatory subunit that was required stoichiometrically for GTP CH stability and activity, as well as for the function of one or more other proteins, some mutations in *Pu* might be expected to exhibit independent effects. The same might be true if *Pu* were responsible for modifying GTP CH and other proteins. However, mutations in such functions should resolve into a discrete number of predictable phenotypes, which we do not observe. We see, instead, a considerable range of mutant alterations. The diversity of functions for which pteridines are employed suggests that the enzymes responsible for their production would exist in a variety of microenvironments suiting individual functions. In this case, GTP CH mutations affecting the behavior of the enzyme in some, but not all, of its cellular environments could also produce diverse and partially independent phenotypes. Since we have not yet found GTP CH antigen or activity in the embryo, we cannot yet choose among the various explanations. We are engaged in two lines of investigation that should resolve the issue. One approach involves a physical characterization of purified GTP CH from several tissues. Another is aimed at defining the embryonic effects of *Pu* mutations and at localizing the *Pu* transcripts and polypeptides in the embryo.

The genetic characteristics of *Pu* parallel those of a number of complex loci in Drosophila. The similarities are, perhaps, illustrated best by comparing the genetics of *Pu* to one of the most thoroughly characterized complex genes in Drosophila, Notch (*N*) (WELSHONS and VON HALLE 1962; WELSHONS 1965, 1971; ARTAVANIS-TSAKONAS, MUSKAVITCH and YEDVOBNICK 1983; KIDD, LOCKETT and YOUNG 1983; GRIMWADE *et al.* 1985). Mutations in *N* result in a host of dominant and recessive phenotypes affecting the viability of embryos

as well as eye facet and wing morphology in the adult. As in the case of *Pu*, some *N* mutations are recessive, homozygous viable and have tissue-specific phenotypes. Other *N* mutations result in lethality. Some *lethal/viable* heteroallelic combinations can survive. Dominant alleles of both *N* and *Pu* are homozygous lethal. *N* deficiency/wild-type heterozygotes have a notched wing phenotype, whereas *Pu* deficiency/wild-type heterozygotes are transiently mutant in eye color. Mutations in Notch show an extremely complex complementation pattern, including many examples of tissue-specific complementation. The functions of Notch and the basis for the assortment of mutant phenotypes and complex complementation patterns are not understood. However, it is important to note that one set of tissue-specific, recessive visible mutations, the facet (*fa*) alleles, are associated with insertions of middle repetitive DNA into what appears to be intron sequences (GRIMWADE *et al.* 1985). Although it is not yet clear why such lesions should produce the *fa* eye phenotype, they almost certainly bear important clues to the complexity of Notch.

BINGHAM and ZACHAR (1985) have another example of a tissue-specific mutation in Drosophila caused by a DNA insertion. They found that $w^{DZL}$, an allele of white resulting from the insertion of a transposon 6 kb 5′ to the start site of the major white transcript, affects transcription in adult heads. The function of the gene in other tissues and in other developmental stages appears to be normal. A tissue-specific defect resulting from the insertion of mobile element DNA has also been found in a Drosophila tropomyosin gene (KARLIK and FYRBERG 1985). In this case, the element inserted into a portion of the gene that is transcribed but variably spliced. The region serves as an exon in adult flight muscle transcripts, but is spliced out of transcripts in larvae and in nonfibrillar adult muscle. The mutant *Ifm(3)3* fails to make the tropomyosin isoform specific to flight muscle. The isoform found in other Drosophila muscle tissue is produced as usual. Some of the complexity of *Pu* could result from insertional events similar to those just described. However, transposon insertions are not the only means by which complex phenotypes and complementation patterns could be generated. Data are rapidly accumulating which indicate that the range of expression and the product repertoires of many eukaryotic genes have been expanded by the use of alternative promoters or 3′ termini or by alternative internal exon splicing (YOUNG, HAGENBÜCHLE and SCHIBLER 1981; BENYAJATI *et al.* 1983; MEDFORD *et al.* 1984; KARLIK *et al.* 1984; BREITBART *et al.* 1985; SHAW, SORDAT and SCHIBLER 1985). Any type of mutation, including single base changes, that occurs within portions of a gene used in a tissue- or stage-specific manner could produce complex phenotypic patterns, as long as other splicing modes or use of other promoters and termination sites were not affected.

The complexity of *Pu* may be generated in any one or all of the above ways. An understanding of the features of this gene that set the stage for tissue-specific phenotypes obviously requires a molecular dissection of wild-type and mutant loci. Accordingly, the Punch region has been cloned (J. PAULUS, R. BOSWELL and J. M. O'DONNELL, unpublished observations), and we are proceeding with a molecular analysis of the locus. Ultimately, however, complex

phenotypes and complementation patterns can only be understood within the context of the structures of the gene products and their subcellular interactions. The data accumulating from our analysis of *Pu* provide a framework within which we can also investigate this feature of gene expression.

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