have not suffered a deletion of the gene is shown by the normal saturation hybridization of the F4+ template inactive DNA with ^{3}H-labeled globin cDNA (Fig. 2). Thus, chromatin from a cell line which does not have the potential to transcribe globin sequences does not have the globin gene in a conformation which allows its fractionation into the template active chromatin fraction.

These results have three important implications. First, they show that an active gene can be localized in the template active fraction of chromatin. Second, the finding that in uninduced FSD-3 cells the globin gene is present in a conformation which allows its fractionation with template active chromatin suggests that the globin gene is switched on somewhat early in differentiation of erythropoietic cells and that prior to its involvement in actual transcription it is activated by a cytoplasmic or nuclear signal. It is not unlikely that this would be true for any inducible gene, that is, a gene which can be turned on by some specific environmental signal such as a hormone, or other chemical agent. This might differ from a differentiation process in which the cells in a lineage must undergo multiple changes in the expressed genetic program and which could, for example, require a round of cell division to achieve these changes (*17*). Finally, the localization of the globin gene in the template active fraction of FSD-3 cell chromatin argues that the target cell for Friend virus is a somewhat differentiated erythroid committed precursor cell. This supports the earlier observation that the virus needs a mature precursor cell to express its oncogenic potency (*18*).

R. BRUCE WALLACE
SHYAM K. DUBE*
JAMES BONNER

Division of Biology, California Institute of Technology, Pasadena 91125

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* Present address: Department of Biological Chemistry, Harvard Medical School, Boston, Mass. 02115.

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Mechanism of Suppression in *Drosophila*: Control of Sepiapterin Synthase at the Purple Locus

Abstract. *The amounts of sepiapterin and red pteridine eye pigments (drosopterins) in* Drosophila melanogaster *are known to be reduced in the purple mutant and restored to normal by a suppressor mutation. We show here that sepiapterin synthase activity is 30 percent of normal in* pr *and* pr^{bw}*, two naturally occurring alleles of purple, and is restored to nearly normal levels by the suppressor* su(s)^2*. A heterozygote of two newly induced alleles of* pr *has even lower enzyme activity (< 10 percent). The sepiapterin synthase activity is proportional to the number of wild-type* pr *alleles in flies when one and two copies of the allele are present and is higher in three- than in two-dose flies. We hypothesize that the purple locus may be a structural gene for sepiapterin synthase in* Drosophila.

The purple mutant of *Drosophila melanogaster* is deficient in pteridines (*1*). The red pteridine eye pigments, drosopterins, are reduced to 30 percent of normal in two alleles of purple, *pr* and *pr^{bw}*, and are restored to normal levels in *su(s)^2; pr* and *su(s)^2; pr^{bw}* (*2*). In addition the sepiapterin pool undergoes changes that parallel those in the drosopterins (*2*). These results indicate that the biochemical defect in purple is in pteridine biosynthesis and that the defect is most probably among the early steps in the pathway.

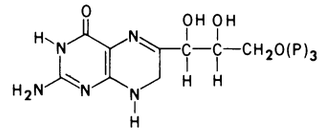
In this report two early enzymatic activities in pteridine biosynthesis, guanosine triphosphate (GTP) cyclohydrolase and sepiapterin synthase, were measured in wild-type, purple, and suppressed purple flies. Each activity has been previously demonstrated in *Drosophila* (*3*, *4*).

Two new alleles of purple were isolated by treating the flies with the mutagen ethylmethane sulfonate. The treated *pr$^+$cn* male flies were crossed with *Sco pr cn/CyO, pr cn^2* females. Flies containing the purple mutation were selected, and stocks of the newly induced *pr* alleles were constructed. One new mutation, *pr^{c4}*, is lethal as a homozygote and behaves as the least functional of all the purple alleles, indicating that the product of the *pr* locus is required for viability of *Drosophila*. White eyes are expected if the purple mutation is extreme (not leaky) since cinnabar is included in these stocks to prevent the synthesis of brown ommochrome eye pigments. The heterozygote, *pr^{c4}/pr*, is distinctly lighter in eye color than *pr/pr*. The second new mutant, *pr^{c5}*, is associated with a translocation between the Y and second chromosome and shows a variegated position effect. The eye color of *pr^{c5} cn/pr cn* is orange like that of *pr cn/pr cn*, but that of *pr^{c4} cn/pr^{c5} cn* is white with yellow flecks. The eye color of *pr^{c4}/pr^{c5}* tends to darken with age.

Guanosine triphosphate cyclohydro-

lase, the first enzyme in pteridine biosynthesis, was measured in *pr*, pr^{bw}, and pr^{c4}/pr^{c5}, and in no case was the enzyme activity less than in the wild type (Table 1).

Dihydroneopterin triphosphate (**1**), the product of GTP cyclohydrolase, is converted to sepiapterin (**2**) by one or more enzymes (*4*) and we next examined this reaction. The separation of sepiapterin from other ^{14}C-labeled compounds is shown in Fig. 1; comparison of the radioactivity in the sepiapterin spot shows that the sepiapterin synthase in two purple mutants, *pr* and pr^{bw}, is reduced to low levels relative to that in the wild type. Furthermore, the presence of $su(s)^2$ restores the enzyme in homozygous pr^{bw} to near normal amounts. This indicates that the metabolic defect in purple mutants is located in one of the steps in the conversion of dihydroneopterin triphosphate to sepiapterin, and that $su(s)^2$ somehow restores this activity. An alternative interpretation could be that sepiapterin is degraded faster in purple mutants and the radioactive sepiapterin therefore failed to accumulate. When we added sepiapterin prior to the reaction to protect the product there was no increase or decrease in



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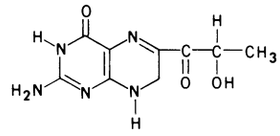

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the amount of radioactive sepiapterin isolated. This alternative is therefore unlikely. Quantitative results from comparison of the sepiapterin synthase activity in these genotypes appear in Table 1. All *pr* alleles are reduced in enzyme activity; and the flies containing the combined alleles pr^{c4}/pr^{c5} have the lowest activity. When other mutants (brown, sepia, prune, $Henna^{r3}$) that are defective in pteridine eye pigments were examined, all had normal levels of sepiapterin synthase. The amount of sepiapterin synthase in $su(s)^2$; pr^{bw} is usually about 90 percent of that in the wild type, but in some instances may be only 50 to 60 percent. The reason for the variability is not known.

Since the sepiapterin synthase activity is associated with the purple locus, we asked if this locus was the structural gene for the enzyme. For another enzyme, xanthine dehydrogenase of *Drosophila*, several loci are involved in determining the activity of the enzyme but only one, the rosy locus, is the structural gene (*5*). Two pairs of genotypes were examined: one had either one or two wild-type alleles of purple and the other pair had either two or three such alleles. The construction of the combinations was done as illustrated in Fig. 2, which shows the ratio of activities from each pair of offspring. The ratio of activities in flies with one wild-type pr^+ to those with two was 0.51 with a standard error of 0.04 in five determinations. The comparison of the pair involving two and three alleles of pr^+ required the comparison of males and females, because sex difference was used to differentiate the two- and three-dose flies. It was estimated that males contain 1.34 the sepiapterin synthase of females, so this ratio was used to adjust the ratio of 1.63 found by comparing the flies with two and three alleles of pr^+; a value of 1.21 was obtained for the adjusted ratio. Four sets of flies were assayed in duplicate to

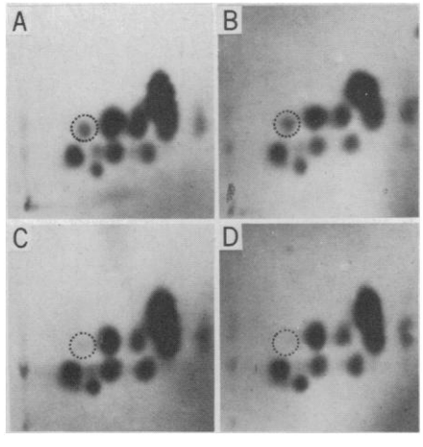

Fig. 1 (left). Separation of sepiapterin by thin-layer chromatography. Sepiapterin synthase assays and two-dimensional chromatographic separation of the products are described in Table 1. Autoradiograms of the developed chromatograms were made with Kodak RP/R-54. The spot that showed a yellow fluorescence is circled to indicate the location of sepiapterin on the chromatogram. The following genotypes of *Drosophila melanogaster* were the source of extracts: (A) Oregon-R, (B) $su(s)^2$; pr^{bw}, (C) pr^{bw}, and (D) *pr*.

a.

Parents $\underline{pr^+}/\underline{pr^+}$ (Ore-R) X $\underline{Tft\ pr^{c4}\ cn}/\underline{pr^+}$ (Ore-R)

$\frac{\underline{Tft\ pr^{c4}\ cn}}{\underline{pr^+}\ \text{(Ore-R)}}$ and $\frac{\underline{pr^+}\ \text{(Ore-R)}}{\underline{pr^+}\ \text{(Ore-R)}}$

one dose of $\underline{pr^+}$ two doses of $\underline{pr^+}$

Ratio $\frac{\text{one dose}}{\text{two doses}}$: 0.562, 0.640, 0.458, 0.471, 0.433

b.

Parents $\underline{pr^+}/\underline{pr^+}$ (Ore-R) X $\underline{Dp(2;Y)G, pr^+}; \underline{pr^+}/\underline{pr^+}$ (Ore-R)

$\underline{pr^+}/\underline{pr^+}$ (Ore-R) and $\underline{Dp(2;Y)G,\ pr^+}; \underline{pr^+}/\underline{pr^+}$ (Ore-R)

two doses of $\underline{pr^+}$ (females) three doses of $\underline{pr^+}$ (males)

Ratio $\frac{\text{three doses}}{\text{two doses}}$: 1.658, 1.919, 1.714, 1.214

Fig. 2 (right). Sepiapeterin synthase levels in flies with one, two, and three doses of pr^+. (a) Genotypes of *Drosophila* and amounts of sepiapterin synthase in flies with one and two doses of pr^+. The mutant allele pr^{c4} was chosen because it appears to have little or no pr^+ function. The dominant mutation, *Tft* (Tuft), is used to follow pr^{c4} to which it is linked. The chromosomes were introduced into an Oregon-R (Ore-R) background. The one- and two-dose flies are sibs and hence were grown in the same culture bottles. (b) Genotypes of *Drosophila* and amounts of sepiapterin synthase in flies with two and three doses of pr^+. The extra dose of pr^+ beyond the normal diploid number of two is inserted in the Y chromosome. The duplication is one part of the insertional translocation, *T(Y;2)G*. Sex serves as a marker in that males have three doses of pr^+ and females have two. The measured ratios of sepiapterin synthase activities are shown for each pair.

obtain the male to female ratio and the three pr^+ to two pr^+ ratio. Statistical analysis indicated that the standard error for the final adjusted ratio was 0.11 and that the 95 percent confidence limits were 0.86 and 1.57. The expected value of 1.5 was not obtained, so the data were examined to determine the probability that the ratio of 1.21 was significantly greater than 1.0. If one assumes a t distribution with 3 degrees of freedom, the probability was only .075 that a ratio of 1.21 would be obtained if 1.0 were the actual mean ratio. Therefore, we conclude that pr^+ causes more sepiapterin synthase to be present in flies with three alleles of pr^+ than with two, but we do not find 1.5 times as much as might be expected. A recent report by Kiger and Golanty (*6*) indicates a relation between guanosine 3′,5′-monophosphate phosphodiesterase activity and gene dose in that the presence of a large duplication resulted in 1.5 times the activity of the diploid, but smaller duplications showed much less of an increase. The use of chromosomal duplication to define a structural gene is not always straightforward. In the case of sepiapterin synthase the ratio of enzyme activity in flies with one dose of pr^+ to two doses of pr^+ was quite close to that predicted for the structural gene, and since the three pr^+ to two pr^+ dose ratio is greater than one, we suggest that the pr^+ locus may be the site for a structural gene for sepiapterin synthase. Further information, such as the finding of a mutation at *pr* locus that results in an amino acid substitution of the enzyme, might confirm this suggestion.

We repeated the measurements of drosopterin contents (*2*) and compared them to the sepiapterin synthase levels in purple, suppressed purple, and wild-type flies. Table 1 indicates that there is a close correlation between the drosopterin pool in the head and the total amount of sepiapterin synthase in these flies. The decrease in drosopterins in *pr* and pr^{bw} agrees well with earlier results (*2*). Suppression of pr^{bw} by $su(s)^2$ restores the pteridine pools and the sepiapterin synthase to normal levels. Although the biosynthetic pathway for drosopterins is not yet understood, these results, along with those of Wilson and Jacobson (*2*), suggest that the compound synthesized from dihydroneopterin triphosphate by the gene product of pr^+ is a key intermediate in the biosynthesis of drosopterins.

Since the transformation of dihydroneopterin triphosphate to sepiapterin involves removal of three phosphate groups as well as oxidation and reduction of the three-carbon side chain it will be of interest to determine which step is affected by the purple mutation.

The suppressor mutant $su(s)^2$ has four target genes whose mutations it suppresses: vermilion, purple, speck, and sable. A change in tyrosine transfer RNA was detected in $su(s)^2$ (*7*) and this change seemed to be related to the reappearance of tryptophan oxygenase activity in $su(s)^2$ *v* (*8*). This tryptophan oxygenase system has been particularly difficult to resolve (*9*, *10*). The speck mutation was shown to be deficient in one of three electrophoretic forms of phenol oxidase and this was restored to normal in $su(s)^2$; *sp* (*11*). From the results in this report we suggest that the sepiapterin synthase is controlled by the purple locus and that it will be useful in elucidating the mechanism of suppression by $su(s)^2$.

Table 1. Quantitative comparison of GTP cyclohydrolase, sepiapterin synthase, and drosopterin (in the head) in purple mutants of *D. melanogaster*. Young adult flies (0 to 3 days old) were homogenized in 50 m*M* piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) (PIPES) buffer (*p*H 7.0) containing 20 percent glycerol in a glass tube and pestle homogenizer at a ratio of 4 ml of buffer to 1 g of flies. The homogenate was centrifuged at 7000*g* for 10 minutes. The resulting supernatant was filtered through Miracloth, and the filtrate was centrifuged at 100,000*g* for 60 minutes. The supernatant was stored at −85°C until used. In some experiments the supernatant obtained at high speed was subjected to fractionation with 40 to 60 percent ammonium sulfate to remove pteridines and low-molecular-weight compounds from the enzyme fraction. The resulting protein precipitate was redissolved in 50 m*M* PIPES, 20 percent glycerol buffer (*p*H 7.0), and used as a source of enzyme. Activity of GTP cyclohydrolase was determined by the method developed by Burg and Brown (*12*). In principle, the determination depends on the measurement of the production of radioactive formate from [8-^{14}C]GTP. Radioactive dihydroneopterin triphosphate was prepared enzymatically by incubation of 100 μM [U-^{14}C]GTP (2.5 μc/25 nmole), 100 m*M* tris-HCl (*p*H 8.3), 100 m*M* NaCl, 10 m*M* EDTA (*p*H 8.0), and pure GTP cyclohydrolase I isolated from *Escherichia coli* (*13*). The incubation was carried out in the dark at 42°C for 60 minutes to achieve conversion of GTP to dihydroneopterin triphosphate. The amount of dihydroneopterin triphosphate produced was determined by the measurement of radioactive formate liberated (*12*). Sepiapterin synthase activity was measured in reaction mixtures having a total volume of 50 μl; 70 m*M* PIPES buffer (*p*H 7.5), 20 m*M* $MgCl_2$, 3.3 m*M*, reduced nicotinamide adenine dinucleotide phosphate, 30 μM [U-^{14}C]dihydroneopterin triphosphate, and *Drosophila* extract. The mixture was incubated in the dark at 42°C for 30 minutes, and then heat-treated at 100°C for 5 minutes with 5 nmole of carrier sepiapterin in each reaction mixture. Next, 0.5 unit of alkaline phosphatase was added to each mixture and incubation was continued at 42°C for 20 minutes. After centrifugation, 40 μl of the resulting supernatant was subjected to thin-layer chromatography. The chromatograms were developed as described (*14*). The sepiapterin spot was punched out (1.1 cm in diameter) and the amount of radioactive material was determined in a scintillation counter in 0.4 percent 2,5-bis-2(5-*t*-butylbenzoxazolyl)-thiophene (BBOT) in toluene. Pteridines from *Drosophila* heads were quantitatively determined fluorometrically as described (*14*).

Genotypes	GTP cyclohydrolase* (units)	Sepiapterin synthase† (units)	Drosopterin (fluorescence unit)
Wild type (Oregon-R)	58	2.53	43
Purple mutants			
pr^{bw}	65	0.78	10
pr	67	0.81	15
pr^{c4}/pr^{c5}	70	0.22	2
Suppressed purple			
$su(s)^2$; pr^{bw}	72	2.43	44

*One unit of enzyme produces 1 pmole of [^{14}C]formate per 30 minutes per milligram (fresh weight). †One unit of enzyme produces 1 pmole of [U-^{14}C]sepiapterin per 30 minutes per milligram (fresh weight) under the standard conditions of assay.

JOHN J. YIM, E. H. GRELL
K. BRUCE JACOBSON
University of Tennessee–Oak Ridge Graduate School of Biomedical Sciences, and *Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830*

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