Dominance for sn-glycerol-3-phosphate dehydrogenase activity in *Drosophila melanogaster*: Evidence for differential allelic expression mediated via a trans-acting effect

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Two second chromosomes (*H31* and *T198*) which when homozygous give relatively low α glycerol-3-phosphate dehydrogenase (GPDH) activity have been isolated from natural populations of *Drosophila melanogaster*. In heterozygotes between *H31* and *T198* and chromosomes bearing standard *Gpdh* alleles there is dominance for GPDH activity and for the amount of GPDH protein. The increased level of activity in the heterozygotes appears to be due to the differential expression of the standard alleles rather than to increased heterodimer activity. Recombination analyses have shown that if the effect is due to a modifier it must be less than 0.04 cM from the *Gpdh* locus.

INTRODUCTION

In diploid species genetically determined electrophoretic enzyme variants at structural gene loci are invariably codominant. Heterozygotes for alleles which encode electrophoretically distinct proteins are expected to produce both forms and, if the enzyme product is a dimer, hybrid enzyme molecules. If the enzyme products of two distinguishable alleles differ in activity the heterozygotes are expected to be of intermediate activity.

These expectations have usually been fulfilled in electrophoretic surveys of a wide range of animals and plants. The majority of exceptions come from studies of inter-specific hybrids in which there is preferential gene expression of one homologous gene over the other (e.g., Castro-Sierra and Ohno, 1968; Wright and Moyer, 1966; Scholl and Anders 1973; Herrera, 1979; Whitt, 1981; Dickinson, Rowan and Brennan, 1984). Within species exceptions are much rarer, although differential allele expression exists for alcohol dehydrogenase (Schwartz, 1971) and alpha-amylase (Chao and Scandalios, 1971) in maize, 6-phosphoglucose dehydrogenase in carp (Klose and Wolf, 1970), peroxidase in rice (Endo, 1971) and for xanthine dehydrogenase (McCarron,

O'Donnell, Chovnick, Bhullar, Hewitt and Candido, 1979) and ribosomal RNA genes (Procunier and Tartof, 1978) in *Drosophila melanogaster*. In the case of alcohol dehydrogenase in maize and xanthine dehydrogenase and the ribosomal genes in *D. melanogaster* detailed genetic analyses show that the differential expression is due to tightly linked cis-acting modifiers. (Schwartz, 1971; Procunier and Tartof, 1978; and McCarron *et al.*, 1979).

We describe in this paper a further example in *D. melanogaster* in which functional electrophoretic variants of soluble sn-glycerol-3-phosphate dehydrogenase (sn-glycerol-phosphate; NAD⁺ oxidoreductase; EC 1.1.1.8; GPDH) are differentially expressed in heterozygotes. The regulatory factor is trans-acting and gives rise to dominance in the steady state level of GPDH in heterozygotes (Gibson *et al.*, 1986).

MATERIALS AND METHODS

The sn-glycerol-3-phosphate dehydrogenase (*Gpdh*) electrophoretic variants, *F* and *S*, used as standards in these experiments were extracted from flies collected from a natural population of *D.*

melanogaster at Cygnet (Tasmania). In addition, two putative *Gpdh* alleles with unusual properties, *T198* and *H31*, were extracted from the Tasmanian populations Tamar and Huonville respectively. In all extractions *Cy0* and *TM6* were used as second and third chromosome balancers (Lindsley and Grell, 1968) and in each stock the third chromosomes were derived from the same populations as the second chromosomes. *Gpdh* variants with similar properties to *T198* and *H31* have been isolated from other populations to be described elsewhere.

The Cygnet and Huonville populations are about 10 km apart in the south of the island and the flies were breeding in waste apple pressings. Tamar is 300 km to the north and there the flies were breeding in discarded grape pressings.

T198 and *H31* (and the other variants with similar properties) were identified in experiments in which up to 300 wild caught male flies from each of the three Tasmanian populations were mated in turn, in single pair cultures, to females homozygous for either *F* or *S* standard alleles (O'Brien and MacIntyre, 1970), which were derived from the previous year's collection of flies at Cygnet. The male parents were electrophoretically typed for GPDH phenotypes and the progenies from male *F/S* heterozygotes discarded. Progenies from cultures where the male parent was homozygous for either *F* or *S* were scored for GPDH phenotypes. A number of pairs of unexpected progenies were obtained in which, for example, both *F* and *S* homozygotes occurred in one set of progeny and only *F* homozygotes in the other progeny from the same male. These were explicable if the male parent was heterozygous for a null activity allele at the *Gpdh* locus. Extraction of the relevant second chromosomes showed this to be the correct explanation (Freeth and Gibson, 1985). However, other progenies were unusual in that putative heterozygotes exhibited abnormal electrophoretic phenotypes. A number of second chromosomes giving rise to these variant phenotypes were extracted and made homozygous; these included *T198* and *H31* which were chosen for further analysis as they differed from each other in electrophoretic mobility. In the experiments described here, the designations *T198* and *H31* represent second chromosomes containing these putative *Gpdh* alleles.

A number of laboratory stocks were also utilised; *Df(2L)GdhA* is an X-ray induced deficiency for clot (*cl*) and *Gpdh* (Grell, 1967) which extends from 25D7-E1 to 26A8-9 covering

about 5cM (Kotarski *et al.*, 1983). Stocks homozygous for *cl.Gpdh^{S.spd^{fg}}* and *cl.Gpdh^{F.spd^{fg}}* were kindly provided by Ross MacIntyre.

The GPDH isozymes and their "Fast" and "Slow" electrophoretic variants were identified after electrophoresis of homogenates of single flies on cellulose acetate membranes. The membranes were soaked in running buffer for 1 hour before use. The running buffer contained Tris (126 mM), citric acid (42 mM) and EDTA-di Na (9.26 mM), pH 6.6. Electrophoresis was carried out for 1 hour and the membranes were stained for GPDH activity in 100 mM Tris/HCl (pH 8.6) solution containing DL- α -glycerophosphate, NAD, MTT, PMS and EDTA (di-sodium salt).

Adult *D. melanogaster* homozygous for either the *F* or *S* *Gpdh* variants exhibit three bands of activity, GPDH-1, GPDH-2 and GPDH-3, ordered in decreasing mobility towards the anode on cellulose acetate membranes. All three isozymes are encoded by the same structural gene (localised to the cytogenetic region 25F-26B), but GPDH-3 is derived from GPDH-1 by a proteolytic cleavage of three amino acids at the COOH-terminus (Niesel *et al.*, 1980; 1982). The minor isozyme, GPDH-2, may arise as a hybrid molecule in tissues where GPDH-1 and GPDH-3 are synthesised (Bewley, Rawls and Lucchesi, 1974). Only GPDH-3 is present in third instar larvae. GPDH is a dimer with a sub-unit molecular weight of 32,000 daltons and heterodimers are formed in *F/S* heterozygotes (Niesel *et al.*, 1980).

Stain intensities of the GPDH isozymes after electrophoresis were measured with a Quick Scan integrating densitometer (Helena Laboratories). The electrophoretic techniques, sample concentrations and staining times were standardised to give a linear relationship between the intensity of the stain and the enzyme concentration in the applied sample (correlation coefficient, 0.97).

For GPDH activity measurements 10 five to six-day old male flies were weighed and ground up at 10 mg/ml buffer (0.1 M potassium phosphate, pH 7.1 containing 10 mM EDTA disodium, and 1.05 mM dithiothreitol). The homogenates were centrifuged at 17,000 g for 40 min at 0°C. The clear supernatant provided a crude extract. Enzyme assays of the reverse reaction were carried out with the methods and buffers described by Bewley, Rawls and Lucchesi (1974). A unit of enzyme activity is defined as 1.0×10^{-4} mole of NAD⁺ reduced per min.

The flies for enzyme assays were reared in 4" vials with 20 ml of medium containing per litre, 10 gm agar, 15 gm sucrose, 35 gm dried yeast,

40 gm malt powder, 30 ml corn syrup, 10 gm high protein powder and 4.5 ml propionic acid.

The amount of GPDH protein was assayed by radial immunodiffusion using the method of Lewis and Gibson (1978). Anti-sera prepared in goats to purified GPDH was a gift from David Sullivan.

RESULTS

(i) Electrophoretic phenotypes

The electrophoretic phenotypes of the *F/F* and *S/S* controls, the homozygous *T198* and *H31* strains and the four heterozygotes between these four lines are shown in fig. 1. Densitometer scans of typical electrophoretic patterns from each of the eight genotypes are shown in fig. 2. These figs show that the *T198* homozygotes are electrophoretically indistinguishable from the *S/S* control, while *H31* homozygotes have an identical phenotype to that of the *F/F* control. In each homozygote there are three bands of activity (GPDH-F1, 2 and 3 or GPDH-S1, 2 and 3) with

the middle band (F2 or S2) being the weakest. The *F/S* heterozygotes give five separate bands of activity. The strongest staining bands are in the positions corresponding to S1 or F3 and F2, which contain heterodimers as well as parental enzyme. The heterozygote *T198/H31* has the same pattern with five bands of activity. Heterozygotes between *H31* and *S* also have five bands of activity but the pattern is distinguishable from that of the usual heterozygote because the strongest staining band is the third from the cathode, i.e. in position S1 or F3. In marked contrast is the pattern shown by the heterozygote between *T198* and *F*. No band is detectable in position S3 (which should have the S3 band of *T198*) and the S2 band is weak. The strongest band is in the position of F1 which is derived from the *F* allele.

Scans of 12 electrophoretic separations of each genotype were quantified and the results (table 1) confirm those derived from the visual appearance of the zymograms. There is no significant difference in the relative band intensities between *F/S* and *H31/T198* heterozygotes ($\chi^2_4 = 6.69$, $p > 0.1$),

Figure 1 Electrophoretic phenotypes of GPDH variants on cellulose acetate membranes: (from left to right): *Gpdh^F/Gpdh^F*, *Gpdh^S/Gpdh^S*, *Gpdh^{H31}/Gpdh^{H31}*, *Gpdh^{T198}/Gpdh^{T198}*, *Gpdh^F/Gpdh^S*, *Gpdh^F/Gpdh^{T198}*, *Gpdh^S/Gpdh^{H31}*.

Figure 2 Densitometer scans of GPDH phenotypes after electrophoresis (Note order of genotypes differs from that in fig. 1.) From left to right the five separate peaks are in the positions of bands S3, S2, S1 or F3, F2 and F1. In normal heterozygotes (c) heterodimer is present in all bands except S3 and F1. (a) $Gpdh^S/Gpdh^S$, (b) $Gpdh^F/Gpdh^F$, (c) $Gpdh^F/Gpdh^S$, (d) $Gpdh^{T198}/Gpdh^{T198}$, (e) $Gpdh^{H31}/Gpdh^{H31}$, (f) $Gpdh^{T198}/Gpdh^{H31}$, (g) $Gpdh^S/Gpdh^{H31}$, (h) $Gpdh^F/Gpdh^{T198}$.

whereas $H31/S$ and $T198/F$ differ significantly from each other ($\chi^2_4 = 253.1$, $p < 0.001$) and from the $H31/T198$ or F/S heterozygotes ($\chi^2_4 = 294.41$, $p < 0.001$, and $\chi^2_4 = 382.56$, $p < 0.001$, respectively). Thus three types of GPDH pattern for heterozygotes are clearly distinguishable after electrophoresis even though homozygotes for $T198$ and $H31$ have the same patterns as the F/F and S/S controls.

(ii) GPDH activities and amount of protein

The level of GPDH activity in each of the homozygotes $T198/T198$ and $H31/H31$ is about half the activity of either the F/F or S/S controls (table 2) which do not differ significantly from each other in activity. The GPDH activity in the F/S or $T198/H31$ heterozygotes does not differ significantly from their respective mid-parental values ($t_{(3)} = 1.7$, $p > 0.1$, $t_{(3)} = 0.42$, $p > 0.5$ respectively).

However in heterozygotes between $H31$ or $T198$ and either of the controls F or S , GPDH activity is significantly higher than the respective mid-parental values ($H31/F$ $t_{(3)} = 13.7$, $p < 0.001$, $H31/S$ $t_{(3)} = 8.9$, $p < 0.01$, $T198/F$ $t_{(3)} = 4.6$, $p < 0.05$ and $T198/S$ $t_{(3)} = 4.3$, $p < 0.05$). In each of these four heterozygotes the mean activity was not significantly different to the level of the parent with the highest activity, i.e. the F/F or S/S controls. In all genotypes reciprocal crosses gave similar results (Gibson *et al.*, 1985). Thus the expression of GPDH activity in these heterozygotes exhibits dominance.

To test the generality of the dominance effect we investigated GPDH activity in heterozygotes between $H31$ and the marker chromosome $cl.Gpdh^S.sd^{fg}$. Heterozygotes between $H31$ and $cl.Gpdh^S.spd^{fg}$ had a mean GPDH activity of 352 ± 10.3 , compared with 342 ± 11.8 for $cl.Gpdh^S.spd^{fg}$ homozygotes. The electrophoretic phenotype of the heterozygote on cellulose acetate membranes was characteristic of $H31/S$.

Assays of flies heterozygous for F , S , $T198$ or $H31$ with $Df(2L)GdhA$ show that in each genotype the level of GPDH activity is about half that of the diploid level (table 3).

Over all genotypes there was a correlation of 0.95 between the amount of GPDH protein measured by radial immunodiffusion and GPDH activity. Heterozygotes between $T198$ or $H31$ and F or S had the same amount of GPDH protein as F/F or S/S homozygotes (table 2). Heterozygotes between $T198$, $H31$, F or S and $Df(2L)GdhA$ had half the amount of GPDH protein as their respective diploid levels (table 3).

Table 1 Mean relative percentage activity in each band ($\pm$ standard errors) calculated from the areas under the curves in the densitometer scans

<i>Gpdh</i> genotype	No. of samples	Band of GPDH activity				
		S3	S2	S1 or F3	F2	F1
<i>S/S</i>	10	25.7 $\pm$ 1.9	8.2 $\pm$ 0.6	66.0 $\pm$ 2.3		
<i>F/F</i>	10			34.8 $\pm$ 0.8	10.5 $\pm$ 0.6	54.7 $\pm$ 1.2
<i>F/S</i>	10	6.9 $\pm$ 0.3	13.4 $\pm$ 0.6	28.0 $\pm$ 0.4	32.5 $\pm$ 0.5	19.1 $\pm$ 0.7
<i>T198/T198</i>	8	17.9 $\pm$ 1.9	9.0 $\pm$ 1.3	73.1 $\pm$ 3.1		
<i>H31/H31</i>	12			29.0 $\pm$ 1.3	12.2 $\pm$ 0.8	58.8 $\pm$ 1.9
<i>H31/T198</i>	19	5.7 $\pm$ 0.6	12.4 $\pm$ 0.5	29.1 $\pm$ 0.4	35.2 $\pm$ 0.4	17.6 $\pm$ 0.3
<i>S/H31</i>	16	23.8 $\pm$ 0.5	14.3 $\pm$ 0.2	39.2 $\pm$ 0.4	20.4 $\pm$ 0.3	2.3 $\pm$ 0.02
<i>F/T198</i>	20	none detected	6.6 $\pm$ 0.4	29.1 $\pm$ 0.7	26.9 $\pm$ 0.3	37.4 $\pm$ 0.9

Table 2 sn-Glycerol-3-phosphate dehydrogenase activities ($\pm$ standard errors) and the amount of CRM relative to that observed in the *S/S* homozygote. Each value is the mean obtained in assays of extracts from four separately prepared cultures. The expected activities given are those if heterozygotes are exactly intermediate between the two parental strains

<i>Gpdh</i> genotype	GPDH activity ($\pm$ standard error)	Expected GPDH activity	Amount of CRM
Controls			
<i>F/F</i>	416.2 $\pm$ 18.1	—	1.0
<i>S/S</i>	418.3 $\pm$ 6.3	—	1.0
<i>F/S</i> †	437.0 $\pm$ 11.6	417.2	1.04
<i>H31/H31</i>	184.9 $\pm$ 5.2	—	0.45*
<i>T198/T198</i>	179.9 $\pm$ 5.9	—	0.44*
<i>T198/H31</i> †	179.5 $\pm$ 7.0	182.4	0.43*
<i>H31/F</i> †	447.9 $\pm$ 10.7	300.6	1.1
<i>H31/S</i> †	421.1 $\pm$ 13.4	301.6	1.0
<i>T198/F</i> †	382.4 $\pm$ 18.4	298.0	0.96
<i>T198/S</i> †	384.4 $\pm$ 19.9	299.1	0.94

† Reciprocal crosses gave similar results.

* Significantly different to standard *S/S*, $p < 0.001$.**Table 3** sn-Glycerol-3-phosphate dehydrogenase activity ($\pm$ standard errors) and the amount of CRM relative to that observed in the *S/S* homozygotes, in flies heterozygous for *Df(2L)GdhA*. Each value is the mean of assays of extracts of flies from four separate cultures

<i>Gpdh</i> genotype	GPDH activity	Expected half diploid value	Amount of CRM
<i>F¹/Df(2L)GdhA</i>	223.6 $\pm$ 8.1	208.1	0.58
<i>S/Df(2L)GdhA</i>	230.7 $\pm$ 10.4	209.1	0.55
<i>H31/Df(2L)GdhA</i>	97.4 $\pm$ 8.4	92.4	0.21*
<i>T198/Df(2L)GdhA</i>	87.4 $\pm$ 5.8	89.9	0.21*

* Significantly different to half the diploid CRM level, $p < 0.001$.

(iii) Larval patterns

The electrophoretic phenotypes of *H31* and *T198* in heterozygotes with *S* or *F* are essentially the same in 3rd instar larvae as in adults (although in larvae only the GPDH-3 isozyme is present). Despite this simpler pattern we have been unable to obtain band separation as clear as that for adults. In 3rd instar larvae GPDH activity exhibits dominance in heterozygotes between the standard alleles and either *H31* or *T198*, a finding which will be reported more fully elsewhere.

(iv) Are *T198* and *H31* Alleles at the *Gpdh* locus?

In the breeding programme used to extract the second chromosomes bearing *T198* and *H31* the variants behaved as alleles at the *Gpdh* locus. If however the differential expression of, for example *T198*, is due to a modifier linked to the *Gpdh* locus (i.e., *mS*) then it would be possible by recombination to separate the modifier from the *S* allele. To test this possibility we investigated the electrophoretic phenotypes of recombinant progeny produced in the crosses *T198/cl.F.spd*♀ $\times$ *cl.F.spd/cl.F.spd*♂ and *H31/cl.S.spd*♀ $\times$ *cl.S.spd/cl.S.spd*♂. In 403 recombinants we have not been able to separate any modifier effect from either *T198* or *H31* and produce a normal *F/S* heterozygote (table 4). As the map distance *cl-Gpdh* is 1.67 cM and *Gpdh-sp^d¹⁸* is 4.65 cM any modifier of *T198* must be less than 0.027 cM, and of *H31* less than 0.037 cM, from the *Gpdh*⁺ locus.

DISCUSSION

The putative alleles *H31* and *T198* are distinguishable from the standard *F* and *S* alleles by their

Table 4 GPDH phenotypes of recombinant genotypes in the *cl-spd^{fs}* region

Chromosome	Marker phenotype		
	GPDH phenotype	<i>cl⁺.spd^{fs}</i>	<i>cl.spd^{fs}⁺</i>
<i>H31</i>	<i>S/S</i>	25	76
	<i>H31/S</i>	52	20
	<i>F/S</i>	—	—
<i>T198</i>	<i>F/F</i>	17	83
	<i>T198/F</i>	84	46
	<i>F/S</i>	—	—

relatively low GPDH activities in homozygotes and by their effect on GPDH activities and electrophoretic phenotypes in heterozygotes with the normal alleles. Homozygotes for the chromosomes bearing *H31* and *T198* produce electrophoretic phenotypes on cellulose acetate membranes indistinguishable from those of standard *F* or *S* homozygotes respectively, and heterozygotes between *T198* and *H31* have the same phenotype as *F/S* heterozygotes. Yet heterozygotes *F/T198* and *S/H31* have phenotypes that differ from each other and from *F/S*. Although the homozygotes *T198/T198* and *H31/H31* each have about half of the GPDH activity of the standard homozygotes, the heterozygotes *T198/F* or *S* and *H31/S* or *F*, have similar activities to the standard homozygotes.

The increased activity of the heterozygotes between *T198* or *H31* and the standard chromosomes is paralleled by an increase in the amount of GPDH protein (CRM) compared to the mid-parental value. These data, and the electrophoretic phenotypes, argue against the idea that the dominance effect is due either to the hybrid enzyme having a higher catalytic efficiency than the homodimers (Fincham, 1966) or to increased amounts of heterodimer. The question thus arises as to whether the expression of both alleles is increased in heterozygotes. The densitometer scans show that in *F/T198* the S₃ band derived from

T198 is too faint to detect and that the heaviest staining band is in the F₁ position derived from *F*. In *H31/S* heterozygotes there is heavy staining in the S₃ and S₁ or F₃ band positions. Based on the average percentage composition of the F₁ band of *F/F* and the S₁ band of *T198/T198*, the F₁ band of *F/T198* would be expected to have about 24 per cent of the total activity of *F/T198*, whereas it has 37 per cent (table 1). Similar calculations show that the S₃ band of the *F/T198* heterozygotes was expected to have 1.2 per cent total activity, whereas no band was detected. Also, in the *S/H31* heterozygotes the S₃ band was expected to be 12 per cent of the total activity, whereas it accounted for 22.1 per cent, and the F₁ band was 3.0 per cent, whereas 4.9 per cent was expected. Thus these data suggest that in the *F/198* and *S/H31* heterozygotes the GPDH-F or GPDH-S enzyme, respectively, is present at higher levels than expected. Assuming that *all* the activity above mid-parental level in, for example, *F/T198* is due to the presence of more product of the *F* allele (and that the product will be present as GPDH-1, GPDH-2 and GPDH-3 in the same proportions as in *F/F*) the expected proportions of activity in each of the five bands is given in table 5. These data and those derived in a similar manner for *S/H31*, show that the observed and expected proportions are not significantly different (*F/T198*, $\chi^2_4 = 2.65$, $p > 0.05$, *S/H31*, $\chi^2_4 = 2.74$, $p > 0.05$).

We have quantified the electrophoretic phenotypes of *F/H31* and *S/T198* heterozygotes after the cellulose acetate membranes have been held in a water bath for 20 min. at 50°C prior to staining. This removes the heat labile isozymes GPDH-2 and GPDH-3 (Bewley *et al.*, 1974; Bewley, 1978) so that the heterozygotes are expected to have three bands of activity derived from GPDH-1. Fig. 3 and table 6 show that in *S/H31* and *F/T198* the homodimer bands, S₁ or F₁ respectively, are twice as intense as the heterodimer bands. In each genotype the relative proportions of the bands suggest that the enzyme produced by the normal allele is present at more than four times

Table 5 The expected percentage activities in heterozygotes *F/T198* and *S/H31* based on the data in tables 1 and 2 and assuming that the increased activity in the heterozygotes is solely due to the *F* or *S* alleles

<i>Gpdh</i> genotype	Band of GPDH activity				
	S ₃	S ₂	S ₁ or F ₃	F ₂	F ₁
<i>F/T198</i>	0.6	7.8	30.8	30.6	26.9
<i>S/H31</i>	14.9	14.4	46.7	21.4	2.6

Figure 3 GPDH electrophoretic phenotypes after cellulose acetate membranes have been held in a water bath at 50°C for 20 min prior to staining: (from left to right): *Gpdh^F/Gpdh^F*, *Gpdh^{H31}/Gpdh^{H31}*, *Gpdh^S/Gpdh^S*, *Gpdh^{T198}/Gpdh^{T198}*, *Gpdh^F/Gpdh^S*, *Gpdh^S/Gpdh^{H31}*, *Gpdh^F/Gpdh^{T198}*, *Gpdh^{H31}/Gpdh^{T198}*.

Table 6 Mean percentage activity remaining in each band ($\pm$ standard error) after cellulose acetate membranes were held in a water bath at 50°C for 20 min prior to staining for GPDH

<i>Gpdh</i> genotype	Number of samples	Band of GPDH activity		
		S1	Heterodimer	F1
<i>S/S</i>	8	29.8 $\pm$ 1.6	53.1 $\pm$ 2.3	17.1 $\pm$ 0.9
<i>T198/H31</i>	7	23.8 $\pm$ 2.4	59.1 $\pm$ 3.4	17.1 $\pm$ 2.0
<i>S/H31</i>	8	67.2 $\pm$ 1.6	32.8 $\pm$ 1.1	n.d.
<i>F/T198</i>	8	2.8 $\pm$ 1.7	37.5 $\pm$ 1.2	59.7 $\pm$ 1.8

n.d. = non-detected.

the level of the enzyme produced by either *H31* or *T198*.

As heterozygotes between *T198*, *H31*, *F*, or *S* and *Df(2L)GdhA* each have half the diploid level of activity and CRM the phenomenon is not due to some compensatory mechanism mediated via promoters of different strengths (as have been found to modify alpha-amylase levels in mice; Schibler *et al.*, 1983).

In summary, the results show that there is an interaction between elements close to the *Gpdh*⁺ locus in *H31* or *T198* and the normal *Gpdh* gene region, which enhances GPDH activity. The modification appears to be trans-acting and results in dominance in the level of enzyme activity and CRM.

We do not yet know the mechanism of this differential gene expression. The increase in GPDH activity could be due to increased transcription from the normal allele or increased *in vivo* stability of the enzyme produced. There is evidence of both kinds of modification of GPDH levels in *D. melanogaster*.

An EMS-induced *Gpdh* allele (*BO-1-5*) exists which has some of the characteristics of *T198* and *H31* (O'Brien and MacIntyre, 1972). *BO-1-5/Df(2L)GdhA* heterozygotes have 12 per cent of normal diploid activity, but when *BO-1-5* is in combination with standard alleles there are normal GPDH levels. Thus *BO-1-5* fails to exhibit dosage compensation. O'Brien and MacIntyre argued that *BO-1-5* is a point mutation which restricts the formation of homodimers but not of heterodimers in combination with the product of normal *Gpdh* alleles. Consistent with this explanation was the evidence that *BO-1-5* showed interallelic complementation with two other EMS mutants. Kotarski *et al.*, (1983) report a similar example in which another EMS induced mutant allele (*Gpdh^{nSP15}*) has a trans effect on standard *Gpdh* alleles. In heterozygotes between either *S* or *F* and *Gpdh^{nSP15}* the GPDH-F or GPDH-S homodimer is reduced in activity. They suggest that the *nSP15* subunit binds with very high affinity to the GPDH-F or *S* monomer to form a heterodimer with no enzymatic activity. However, mechanisms of these kinds would not fit our data as the increased GPDH activity in heterozygotes between either *T198* or *H31* and normal alleles does not appear to be due to increased heterodimer activities.

Natural populations contain modifiers which reduce the activity of GPDH by changing the rate of synthesis of the enzyme (Bewley, 1981 and Shaffer and Bewley, 1983). The modifiers act as cis-regulators and are closely linked to the *Gpdh*⁺ locus. A trans-acting dominant modifier, which acts by lowering GPDH degradation rates resulting in an increase in relative stability, has been described by King and McDonald (1983). However, this modifier is located on the third chromosome, whereas the structural gene *Gpdh*⁺ is on the second chromosome. The mechanism by which the *in vivo* stability of GPDH is increased in this case has not been described, but it is observed in the thorax and not in the abdomen. It is also known that the modifier has similar effects on the level of another abdominal protein. We are investigating whether *T198* or *H31* affect other proteins.

Genetic variation in eukaryotes producing regulatory effects similar to those we have found

may act via post-translational modification (Paigen, 1979). It will be interesting to test whether the trans-regulatory effect in heterozygotes between *H31* and *T198* and standard alleles is via transcriptional (Nevins, 1983) or post-translational mechanisms.

It is possible that *T198* and *H31* are structural variants at the *Gpdh*⁺ locus, as mutations at the structural gene are known to alter the intracellular degradation rate of GPDH, and to give rise to different steady state levels (Bewley *et al.*, 1980). However there is strong evidence that GPDH activity in *D. melanogaster* is controlled by a complex of regulatory and structural components which segregate as a unit (Wilkins, Shaffer and Bewley, 1982; Shaffer and Bewley, 1983) and the factor which distinguishes *T198* and *H31* from the standard alleles might be located within this complex. Genetic mapping of the *H31* and *T198* variants should provide information useful in future investigations of their DNA sequences. It is already clear that any modifier is effectively linked to the structural gene locus so that the electrophoretic phenotypes of *H31/S* and *T198/F* heterozygotes are excellent population markers for an unusual regulatory phenomenon.

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