GENETIC LOCALIZATION AND BIOCHEMICAL CHARACTERIZATION OF A *TRANS*-ACTING REGULATORY EFFECT IN DROSOPHILA

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Manuscript received October 20, 1982
Revised copy accepted May 16, 1983

ABSTRACT

A region-specific, *trans*-acting regulatory gene that alters *in vivo* protein levels of α-glycerophosphate dehydrogenase (α-GPDH) has been mapped to position 55.4 on the third chromosome of *Drosophila melanogaster*. The gene has been found to affect the *in vivo* stability of α-GPDH in adult thoracic tissue but has no effect on α-GPDH levels in the abdomen. Although no other thoracic proteins were found to be influenced by the locus, it appears to modify the level of one additional abdominal protein. The action of the gene over development and its possible mode of control are discussed.

A substantial body of evidence has accumulated over the last several years indicating that there are eukaryotic genes that influence the timing or expression of the products of other nonlinked loci (RAWLS and LUCCHESI 1974; LUSIS and PAIGEN 1975; BOUBELIK *et al.* 1975; ABRAHAM and DOANE 1978; SCANDALIOS *et al.* 1980). Although such genes have been operationally defined as "regulatory," it is recognized that there are a variety of molecular mechanisms by which they may exert their control (McDONALD 1983; MACINTYRE 1982). In Drosophila, such regulatory genes and their controlled "producer genes"[2] have, in some instances, been associated with different chromosomes (McDONALD and AYALA 1978a; LAURIE-AHLBERG *et al.* 1980; TEPPER *et al.* 1982; HORI *et al.* 1982). One producer gene recently shown to be subject to this type of interchromosomal control is *α-gpdh* in *D. melanogaster* (2-17.8, GRELL, JACOBSON and MURPHY 1965).

α-gpdh codes for the soluble sn-glycerol-3-phosphate dehydrogenase (GPDH:NAD^+ oxidoreductase; EC 1.1.1.8), an enzyme known to play a key role in the insect's cytosol-mitochondrial shuttle system (SACTOR 1965). An α-glycerophosphate dehydrogenase (α-GPDH) regulatory effect that alters the level of α-GPDH activity has recently been associated with the third chromosome in *D. melanogaster* (LAURIE-AHLBERG *et al.* 1980; WILSON and McDONALD 1981). We report here the results of a genetic localization and biochemical

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[2] Loci that specify cellular products directly involved in metabolic or structural functions (BRITTEN and DAVIDSON 1969; HEDRICK and McDONALD 1980).

characterization of a region-specific, *trans*-acting gene that modifies the levels of α-GPDH in *D. melanogaster*.

MATERIALS AND METHODS

Drosophila strains

A wild-type strain (*F2*) made completely homozygous for the first, second and third chromosomes was derived from a single wild-caught male collected from a Napa County, California, population (McDONALD, ANDERSON and SANTOS 1980). A substituted strain (*F2, MM3*) was constructed to have the same second (and thus the same *α-gpdh* genotype) and *X* chromosome constitution as the wild-type strain but homozygous for the multiply marked third chromosome carrying roughoid (*ru*, 3-0.00), hairy (*h*, 3-26.5), thread (*th*, 3-43.2), curled (*cu*, 3-50.0), stripe (*sr*, 3-62.0), ebony-sooty (e^s, 3-70.7) and claret (*ca*, 3-100.7). Our method of substituted strain construction utilizes the balancer stock Cy/Bl^2, $SbSer/e^{11}$ and has been fully described earlier (McDONALD and AYALA 1978b). An α-GPDH CRM^- (*i.e.*, lacking detectable levels of α-GPDH cross-reacting material) strain (N-5-4) was kindly provided by GLENN BEWLEY (LEE, NIESEL and BEWLEY 1980).

Biochemical techniques

Enzyme activity assay: α-GPDH activity was measured spectrophotometrically according to a modification of the procedures of McDONALD and AVISE (1976). Whole fly extracts of ten adults (4–8 days posteclosion), early pupae (160–180 hr postoviposition; eyes and wing pads not visible), late pupae (150–160 hr postoviposition; eyes and wing pads visible) or third instar larvae (135–145 hr after hatching; "roaming stage" with guts visibly free of media) were homogenized in 0.5 ml of 100 mM Tris-HCl (pH 8.6), 8 mM EDTA.

Adult tissue-specific expression of α-GPDH, alcohol dehydrogenase (ADH) and phosphoglucose isomerase (PGI) enzyme activities were investigated by homogenizing adult thorax and abdomen sections separately (20 sections/ml). All homogenates were centrifuged at 5° at 12,000 × *g* for 20 min, and the supernatant was recovered for enzymatic analysis. α-GPDH activity was measured on either a Beckman ACTA II (adding 100 μl of crude extract to 900 μl of the reaction mix) or a Du-8 spectrophotometer (via an "improved enzyme assay" that extended the period of time for which the reaction (ΔOD) was linear: 10 μl of crude extract to 990 μl of the reaction mix). The reaction mix is 1 mM NAD^+, 90 mM D,L-α-glycerophosphate made up in 100 mM Tris-HCl, (pH 8.6). ADH and PGI activites were monitored on a Du-8 spectrophotometer by previously published techniques (McDONALD and AVISE 1976; AVISE and McDONALD 1976). All flies examined in this study were determined to be insignificantly different in weight per individual and milligrams of protein per individual.

Electrophoresis: Polyacrylamide gel electrophoresis was carried out in 7% gels according to the method of SMITH (1968). Gels were specifically stained for α-GPDH as described by AYALA *et al.* (1972).

Gel sieving: Polyacrylamide gel sieving was as described by JOHNSON (1975) using bovine hemoglobin as a standard.

Isoelectric focusing: Isoelectric focusing was performed according to LKB, Inc. manual no. 1804. Samples were homogenized in distilled H_2O (six adult ♂♂/0.1 ml) and focused in a thin layer acrylamide gel (72 × 24 cm) containing an ampholine pH gradient (pH 5.5–8.5). The gradient was set up in the long direction to maximize separation. Focusing was at 11 watts of constant power for 2 hr. The gel was stained for α-GPDH activity and sliced into strips, and the bands were scanned on a Beckman Du-8 spectrophotometer at 560 nm. The relative areas under each α-GPDH activity peak were computed for each sample.

α-GPDH antisera. α-GPDH antisera were prepared from purified α-GPDH (40–60% ammonium sulfate cut followed by electrophoretic separation in a 7% polyacrylamide gel). α-GPDH bands were cut out of the polyacrylamide gel, homogenized in distilled H_2O and subcutaneously injected into New Zealand white rabbits. The antisera were otherwise prepared according to the method of McDonald *et al.* (1977).

The specificity of the prepared α-GPDH antisera was verified by the radioimmunological procedures of ANDERSON and McDONALD (1981). The results presented in Figure 1 demonstrate that more than 80% of the precipitable [^{14}C] counts associate with α-GPDH protein separated on a 7% sodium dodecyl sulfate polyacrylamide gel.

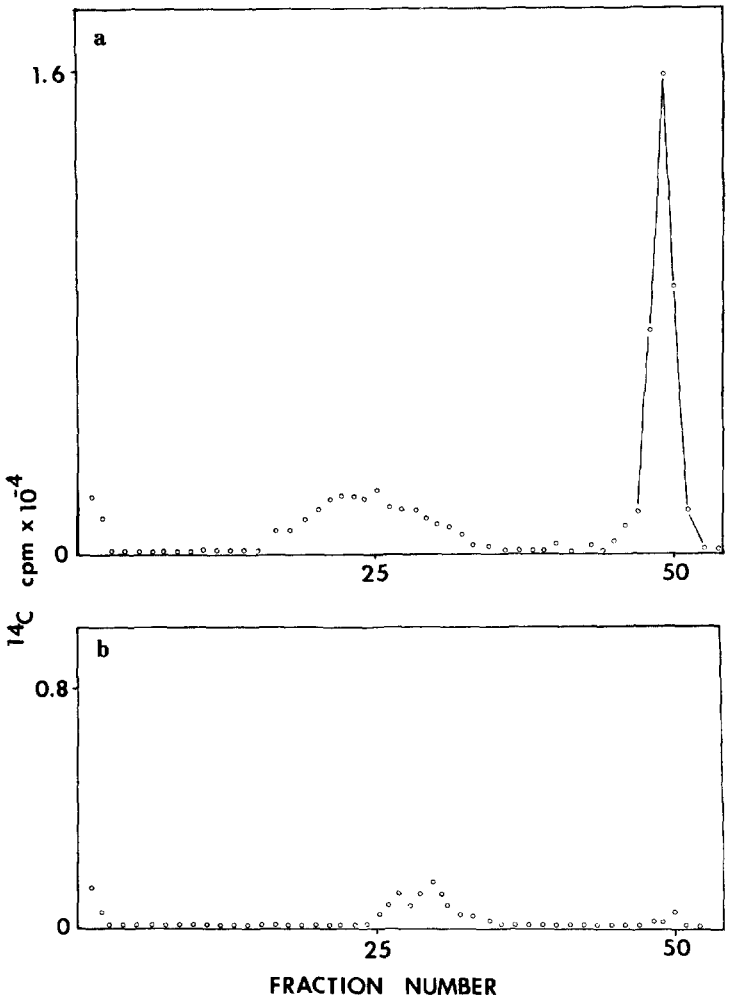

FIGURE 1.—Recovery of [^{14}C] counts per minute from immune precipitates after SDS gel electrophoresis. a, α-GPDH CRM$^+$ crude extract precipitated with anti-α-GPDH sera. The majority of the counts associate with a protein of M_r 33,000, which corresponds to previously determined values for the α-GPDH monomer. b, α-GPDH CRM$^-$ (N-5-4) crude extract precipitated with anti-α-GPDH sera.

In vivo *estimates of relative α-GPDH synthesis rates* (ks): The rate of incorporation of [^{3}H]-labeled amino acids into α-GPDH was used as a measure of the relative rate of the protein's synthesis according to the procedures of ANDERSON and MCDONALD (1983). Briefly, adult male Drosophila were fasted for 4 hr at 25° under conditions of low humidity (25%) and then placed in shell vials containing filter paper tabs (Whatman 1) that were saturated with a mixture of [^{3}H]L-amino acids (25 μl of 1.0 mCi/mol, New England Nuclear) and 3% sucrose in distilled H_2O (250 μl). At sequential 2-hr intervals, flies were removed from the label and homogenized in 100 mM Tris-HCl (pH 8.6) (45 mg of net weight adult ♂♂/ml buffer). The homogenate was centrifuged (10 min in Eppendorf microfuge), and the supernatant was recovered and divided into four 20-μl replicate aliquots to which 20 μl of α-GPDH antisera were added. After 10–20 hr of incubation (5°), 45 μl of goat antirabbit IgG (Research Products International) were added and incubated for an additional 1.5 hr (5°) to aid in the precipitation of the antigen-antibody complex. After centrifugation, the pellet was resuspended in buffer and repelleted three successive times in order to

wash away trapped, nonspecific counts. The final pellet was dissolved in scintillation fluid (Bray 1960), and radioactivity was quantified in a Packard model 320 scintillation spectrophotometer. Incorporation of counts into α-GPDH was linear over 8 hr. Relative synthesis rate was determined by fitting the data points (counts per minute × 10^{-4} *vs.* time in hours) to a line by least squares analysis (correlation coefficient ≥ 0.95) and calculating the slope (*ks*).

Rate of incorporation into total protein was determined analogously on the same homogenates by trapping trichloroacetic acid (TCA) precipitable counts (5% TCA at 4° for 30 min) on a Millipore filter (pore size 0.45 μm) and scintillation in 5 ml of a toluene fluor [16 g (New England Nuclear) Omni-fluor per 3.8 liters of toluene].

In vitro *estimates of relative α-GPDH stability* (kd): Adult male (4–8 days posteclosion) extracts (30 flies/ml of Tris-HCl, pH 8.6) were prepared as previously described. Percent loss of α-GPDH activity at 25° was monitored at 4- to 8-hr intervals over a period of 24 hr. Under these conditions, rate of activity loss is linear for approximately 13 hr. Relative degradation rate (*kd*) is the slope of the line (natural log of relative α-GPDH activity *vs.* hours) as determined by least squares analysis of the data points taken over 0–13 hr.

RESULTS

A gene that can alter α-GPDH activity has been mapped to a specific position (55.4) on chromosome III: To identify and localize naturally occurring α-GPDH modifier gene variation, a series (three) of third chromosomes were extracted from a natural population (McDonald Ranch, Napa County, California) and combined with a single wild second chromosome (designated *F2*). The relative α-GPDH activity of these strains were determined and contrasted to a strain having the same wild second (*F2*) and *X* chromosome as the previously described stocks but homozygous for the recessively marked third chromosome *MM3* (see MATERIALS AND METHODS). Of the wild third chromosomes surveyed, one (designated *+3*) was found to be asociated with significantly higher ($P < 0.001$, Student's *t*-test) α-GPDH activity than *MM3*. Moreover, the higher activity associated with *+3* was found to be dominant to the *MM3*-associated activity (*F2, +3* = 1.36 units; *F2, MM3/+3* = 1.33 units; *F2, MM3* = 1.00 units).

To map the location of the third chromosome gene or genes responsible for this third chromosome regulatory effect, a backcross was made between the *F2, MM3/+3* heterozygote and the *F2, MM3* marker stock, and the recombinant progeny were scored for visible phenotype and α-GPDH activity (Figure 2). Since a multiple fly homogenate was required for the measurement of α-GPDH activity, it was necessary to pool recombinant classes into ten-fly samples. The mapping experiment proceeded, therefore, in a stepwise fashion such that the regulatory effect was first associated with a broad chromosomal region and then more precisely localized to a specific position within that region.

The results of this stepwise mapping procedure are presented in Table 1. Flies of the *ru . . . h* phenotype were found to have α-GPDH activity levels significantly higher than the low activity *MM3* parental types. On the basis of this, we concluded that the regulatory effect is not associated with the left end of the third chromosome (0-26.5, *cf.* Fig. 3). Recombinants of the *sr . . . e^s* and *th . . cu* phenotypes were also found to have significantly higher α-GPDH activities than *ru . . . cu . . . ca* flies (*F2, MM3*), thus eliminating the regulatory effect from the regions 62.0–70.7 and 43.2–50.0, respectively. Recombinants of

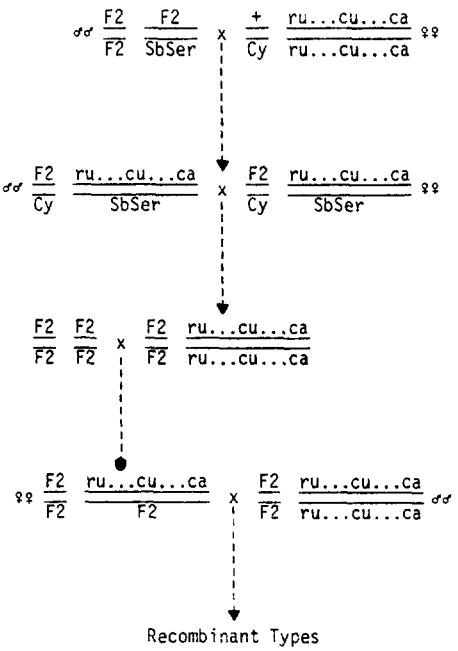

FIGURE 2.—Genetic construction of *F2, MM3* and production of recombinant types (see text for details).

three phenotypes, *h . . . e*^{s}, *th . . . e*^{s} and *cu . . . sr*, were found to have α-GPDH activities insignificantly different from the *ru . . . cu . . . ca* parentals (*F2, MM3*), allowing us to place the regulatory effect within that region common to all three of these types, *i.e.*, between 50.0 and 62.0.

To pinpoint the location of the regulatory effect, we next carried out a recombination analysis between the regulatory effect and the two marker loci that border the area of interest (*i.e.*, *cu* and *sr*). Since the relative α-GPDH activity of each ten-fly homogenate is additive (data not shown) and, thus, the result of the ratio between flies with and without the recombined regulatory effect, we were able to estimate the position of the locus associated with the regulatory effect relative to each recombinant phenotype according to the following equation:

$$p = \frac{\text{activity wild type} - \text{activity of visible recombinant type}}{\text{activity wild type} - \text{activity of } cu\,..\,sr \text{ parental type}}$$

where p is the fraction by which the *cu - sr* interval (12 map units) must be multiplied in order to arrive at the distance of the α-GPDH regulator gene from the locus coding for the particular visible recombinant being scored (*i.e.*, *cu* or *sr*). Based upon enzymatic analyses of more than 200 *cu . . . +* and *+ . . . sr* recombinants, we were able to position the regulatory effect to 55.4 ± 0.3 on chromosome *III* (Figure 3). Henceforth, we will refer to this locus as "regulatory gene 3-55.4." The upper case $R^{3\text{-}55.4}$ will designate the dominant, high α-GPDH activity regulatory allele originally carried on the *+3* third chromo-

TABLE 1

Relative α-GPDH activities of recombinant and parental phenotypes

Visible phenotype	Relative α-GPDH activity	Significance*
ru . . . cu . . . ca	1.00	
+ . . . + . . . +	1.33	P < 0.001
ru . . . h	1.30	P < 0.01
h . . . e^{s}	1.10	N.S.
th . . . cu	1.21	P < 0.001
th . . . e^{s}	1.11	N.S.
cu . . . sr	1.03	N.S.
sr . . . e^{s}	1.19	P < 0.001

* Statistical significance based on Student's *t*-test comparison with the *ru . . . cu . . . ca* control. N.S. = nonsignificant, P > 0.05.

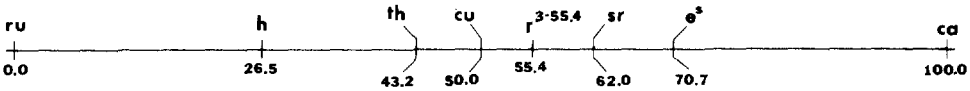

FIGURE 3.—Map of *F2, ru . . . cu . . . ca* third chromosome with $r^{3\text{-}55.4}$ indicated.

some extracted from the wild; the lower case $r^{3\text{-}55.4}$ will designate the recessive, low α-GPDH activity regulatory allele originally carried on the *MM3* marker chromosome.

The α-GPDH activity differences associated with the $R^{3\text{-}55.4}$ *and* $r^{3\text{-}55.4}$ *alleles can be accounted for by differences in* in vivo *levels of α-GPDH cross-reacting material:* To explore the biochemical/molecular basis of the regulatory effect localized to the $R^{3\text{-}55.4}$ locus, we constructed a homozygous stock genetically identical with *F2, +3* except for a small region (~12 map units) of the third chromosome containing the $r^{3\text{-}55.4}$ allele and bordered by the recessive markers *cu* and *sr.* For convenience, this strain will hereafter be referred to as *F2,* $r^{3\text{-}55.4}$. The *F2, +3* will analogously be designated *F2,* $R^{3\text{-}55.4}$ in order to call attention to the fact that the two strains genetically differ only at or immediately around the $R^{3\text{-}55.4}$ locus.

As mentioned earlier, the relative α-GPDH activities of *F2,* $r^{3\text{-}55.4}$ and *F2,* $R^{3\text{-}55.4}$ were 1.00 to 1.33, respectively. Under the "improved assay conditions" described in MATERIALS AND METHODS, this ratio increased to 1.00 to 1.66. The results of radioimmunoassay studies indicate that this activity difference is the result of differences in levels of α-GPDH cross-reacting material (α-GPDH CRM) (Table 2).

The regulatory effect of the $R^{3\text{-}55.4}$ *locus is not ubiquitous but does modify the level of at least one other soluble protein in addition to α-GPDH:* The results of the mapping studies indicate that the α-GPDH activity differences originally observed between our *F2, +3* and *F2, MM3* strains are wholly attributable to genetic differences at the $R^{3\text{-}55.4}$ locus. This fact does not, of course, imply that $R^{3\text{-}55.4}$ is the only third chromosome locus capable of modifying α-GPDH activity. One or more additional α-GPDH regulatory loci may exist on the third chromosome but may not have been genetically variable between the wild and recessively marked chromosomes chosen for analysis in this study. In addition,

TABLE 2

α-GPDH activity and CRM levels in adults expressed relative to strain F2, $r^{3\text{-}55.4}$ *as a control*

Strain	Whole adult		Thorax		Abdomen	
	Activity	CRM	Activity	CRM	Activity	CRM
F2, $R^{3\text{-}55.4}$	1.66**	1.51**	1.55**	1.55**	1.01	1.02
F2, $r^{3\text{-}55.4}$	1.00	1.00	1.00	1.00	1.00	1.00

** Highly significant according to comparison by Student's *t*-test ($P < 0.001$).

it is also possible that the $R^{3\text{-}55.4}$ locus either directly or indirectly influences gene products other than α-GPDH. This latter possibility was explored in two ways.

First, strains *F2,* $R^{3\text{-}55.4}$ and *F2,* $r^{3\text{-}55.4}$ were compared with regard to the activities of two other abundant Drosophila enzymes, ADH and PGI. Like α-GPDH, both of these enzymes are coded for by producer genes located on the second chromosome [*Adh,* 55.1; *Pgi,* 58.6; (O'BRIEN 1980)]. The results, presented in Table 3, demonstrate that both ADH and PGI activities are unaffected by the $R^{3\text{-}55.4}$ locus. The regulatory effect of $R^{3\text{-}55.4}$ is thus not ubiquitous.

In a second study, total soluble protein from *F2,* $R^{3\text{-}55.4}$ and *F2,* $r^{3\text{-}55.4}$ was electrophoresed in a 7% polyacrylamide gel and the intensities of the Coomassie blue-staining bands were compared. The results (Figure 4) demonstrate that the level of at least one other rapidly migrating protein is influenced by the $R^{3\text{-}55.4}$ locus. This protein does not display α-GPDH activity nor does it cross-react with α-GPDH antisera (data not shown). Further biochemical characterization of this protein is presently underway.

The regulatory effect of the $R^{3\text{-}55.4}$ *locus on α-GPDH is region specific:* To determine whether the regulatory effect of $R^{3\text{-}55.4}$ is region specific, *F2,* $R^{3\text{-}55.4}$ and *F2,* $r^{3\text{-}55.4}$ adults were dissected into abdominal and thoracic sections. α-GPDH activity assays of these sections demonstrate that the effect of the $R^{3\text{-}55.4}$ locus on α-GPDH levels is limited to the thorax (Table 2). Although α-GPDH activity is also present in abdominal tissue, no significant difference in levels were found to exist between *F2,* $R^{3\text{-}55.4}$ and *F2,* $r^{3\text{-}55.4}$ flies.

In contrast, polyacrylamide electrophoresis of total soluble protein from each tissue section demonstrated that the regulatory effect of $R^{3\text{-}55.4}$ on the rapidly migrating regulated protein described earlier is limited to the abdomen. In fact, we were unable to detect the presence of this protein in thorax tissue at all.

The allelic α-GPDH regulatory effect of the $R^{3\text{-}55.4}$ *locus is reversed during development:* The α-GPDH activity and CRM relationship between *F2,* $R^{3\text{-}55.4}$ and *F2,* $r^{3\text{-}55.4}$ flies reverses during development (Table 4). *F2,* $R^{3\text{-}55.4}$ flies have 25% lower α-GPDH levels than *F2,* $r^{3\text{-}55.4}$ in larvae but 60% higher levels in adults. A densitometric scan of adult α-GPDH separated by isoelectric focusing is presented in Figure 5. Three isozymic forms designated α-GPDH-1, α-GPDH-2 and α-GPDH-3 are apparent. As a control, a sample of an α-GPDH CRM^-

TABLE 3

ADH and PGI activities

Strain	Thorax		Abdomen	
	ADH	PGI	ADH	PGI
F2, $r^{3\text{-}55.4}$	40.7 ± 1.1	20.4 ± 1.2	41.7 ± 0.9	13.6 ± 1.5
F2, $R^{3\text{-}55.4}$	41.7 ± 0.9	23.2 ± 0.4	43.0 ± 1.1	15.4 ± 0.6

Activities are expressed as $\Delta OD/min \times 10^3 \pm$ S.E.

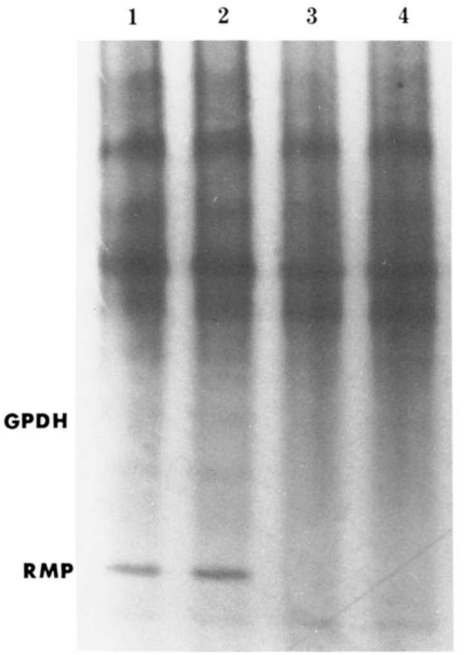

FIGURE 4.—Polyacrylamide gel analysis of *F2*, $r^{3\text{-}55.4}$ and *F2*, $R^{3\text{-}55.4}$ extracts. The region of α-GPDH migration, as determined by activity stain, is indicated (GPDH). In lanes 1 and 2, which contain *F2*, $r^{3\text{-}55.4}$ abdominal extract, the rapidly migrating protein (RMP) is represented. In lanes 3 and 4, which contain *F2*, $R^{3\text{-}55.4}$ abdominal extract, RMP is not detected.

strain (N-5-4, *cf.* LEE, NIESEL and BEWLEY 1980) was run on this same gel, and, as expected, no α-GPDH active bands were detected. Also run on the same gel was a sample of larval protein. The larval samples are clearly distinguished from adult in that all α-GPDH activity is associated with a single band (α-GPDH-3). This stage-specific banding pattern has been previously observed (NIESEL and BEWLEY 1981; NIESEL *et al.* 1982; WRIGHT and SHAW 1969). The pI's determined by us via isoelectrifocusing (α-GPDH-1 = 5.8; α-GPDH-3 = 6.2) are nearly identical with those previously determined by NIESEL *et al.* (1982).

The quantitative distributions of α-GPDH activity into the three isozymic forms is presented in Table 5. The results show that no significant difference in the relative distribution of α-GPDH into the three isozymic forms was apparent between *F2*, $R^{3\text{-}55.4}$ and *F2*, $r^{3\text{-}55.4}$ in pupae and adults. A fraction (14%)

TABLE 4

α-GPDH activity and CRM levels over development expressed relative to strain F2, $r^{3\text{-}55.4}$

Life stage	Strain	Activity	CRM
Adult	*F2*, $R^{3\text{-}55.4}$	1.66**	1.51**
	F2, $r^{3\text{-}55.4}$	1.00	1.00
Late pupae	*F2*, $R^{3\text{-}55.4}$	1.28**	1.30**
	F2, $r^{3\text{-}55.4}$	1.00	1.00
Early pupae	*F2*, $R^{3\text{-}55.4}$	1.31**	1.27**
	F2, $r^{3\text{-}55.4}$	1.00	1.00
Larvae	*F2*, $R^{3\text{-}55.4}$	0.76**	0.70*
	F2, $r^{3\text{-}55.4}$	1.00	1.00

* $P < 0.01$ level of significance.
** $P < 0.001$ level of significance.

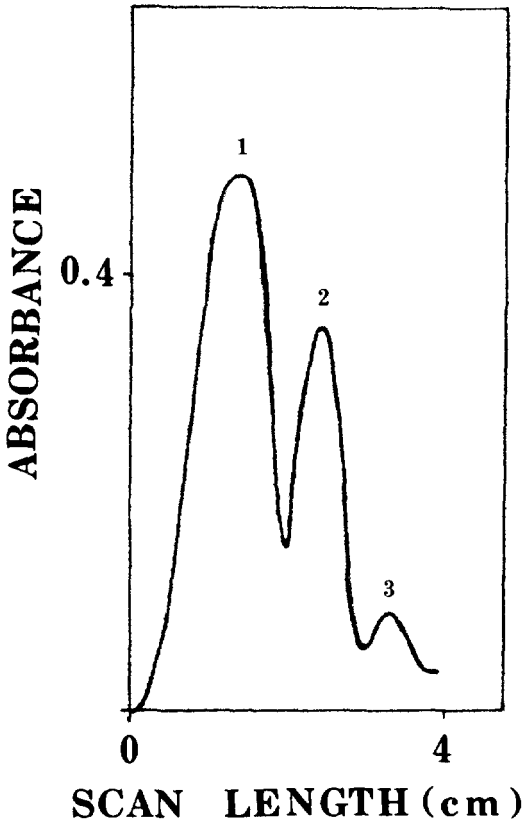

FIGURE 5.—Spectrophotometric scan of an adult showing the three isozyme forms of α-GPDH. (1) α-GPDH-1, (2) α-GPDH-2, (3) α-GPDH-3.

of total α-GPDH in *F2*, $r^{3\text{-}55.4}$ third instar larvae was found to exist as α-GPDH-1 and α-GPDH-2, whereas only α-GPDH-3 activity was detectable in *F2*, $R^{3\text{-}55.4}$ larvae. This latter result is an artifact of the low levels of α-GPDH in *F2*, $R^{3\text{-}55.4}$ larvae. When gels are "overloaded" with *F2*, $R^{3\text{-}55.4}$ extract, all three

TABLE 5

The isozymic distribution of α-GPDH in larval, pupal and adult tissues

Life stage	Strain	α-GPDH-1	α-GPDH-2	α-GPDH-3
Adult	*F2*, $R^{3\text{-}55.4}$	70.94 ± 3.68	22.12 ± 3.17	6.51 ± 1.87
	F2, $r^{3\text{-}55.4}$	76.78 ± 2.19	19.42 ± 2.82	3.78 ± 1.49
Late pupae	*F2*, $R^{3\text{-}55.4}$	59.04 ± 2.37	24.35 ± 1.50	16.6 ± 2.83
	F2, $r^{3\text{-}55.4}$	57.18 ± 3.41	31.16 ± 2.77	11.67 ± 3.09
Early pupae	*F2*, $R^{3\text{-}55.4}$	0	0	100.0
	F2, $r^{3\text{-}55.4}$	0	0	100.0
Larvae	*F2*, $R^{3\text{-}55.4}$	0	0	100.0
	F2, $r^{3\text{-}55.4}$	1.30 ± 1.30	12.91 ± 5.54	85.79 ± 6.47

Results are expressed as percent total area ± S.E.

isozymic forms become apparent in the same relative proportion as found in *F2*, $r^{3\text{-}55.4}$ larvae. In general, then, the relative difference in α-GPDH levels associated with the $^{3\text{-}55.4}$ and $r^{3\text{-}55.4}$ alleles are not attributable to the differential distribution of α-GPDH isozymic forms.

The action of the $R^{3\text{-}55.4}$ locus on α-GPDH activity does not involve detectable changes in protein conformation: Evidence has recently been presented that naturally occurring modifier gene variants exist that can alter the conformation and/or charge of specific Drosophila proteins (*eg.*, JOHNSON, FINNERTY and HARTL 1981). Although we were unable to detect evidence of such $R^{3\text{-}55.4}$ mediated α-GPDH modification via standard 7% polyacrylamide gel electrophoresis or isoelectric focusing, we decided to explore the question further by subjecting *F2*, $R^{3\text{-}55.4}$ and *F2*, $r^{3\text{-}55.4}$ extracts to the gel sieving techniques of JOHNSON (1975). The results presented in Figure 6 indicate no significant effect of the $R^{3\text{-}55.4}$ locus on α-GPDH net charge (*Mo*) or conformation (*Kr*).

The allelic α-GPDH regulatory effect of the $R^{3\text{-}55.4}$ locus is not due to differences in α-GPDH synthesis rates: The results demonstrate that allelic differences at the $R^{3\text{-}55.4}$ locus result in an approximately 60% difference in the steady-state level of α-GPDH protein on a whole fly basis. These differences may be the result of the differential synthesis and/or degradation rate of α-GPDH protein. The results of radioimmunological determinations of relative *in vivo* synthesis rates (*ks*) of strains *F2*, $R^{3\text{-}55.4}$ and *F2*, $r^{3\text{-}55.4}$ are presented in Table 6. The data demonstrate that the differences in steady-state levels of α-GPDH protein that exist between these strains are not the result of differential rates of protein synthesis.

The allelic α-GPDH regulatory effect of the $R^{3\text{-}55.4}$ locus is due to differences in α-GPDH degradation rates: Relative degradation rate (*kd*) can be calculated at steady state by the equation $kd = ks[E]$, where E is the relative enzyme concentration existing between strains at steady state. Table 6 presents the calculated *in vivo* degradation rates for *F2*, $r^{3\text{-}55.4}$ and *F2*, $r^{3\text{-}55.4}$ based on the relative CRM and *ks* values. The results indicate that the $R^{3\text{-}55.4}$ locus regulates relative levels of α-GPDH protein by modifying relative α-GPDH stability.

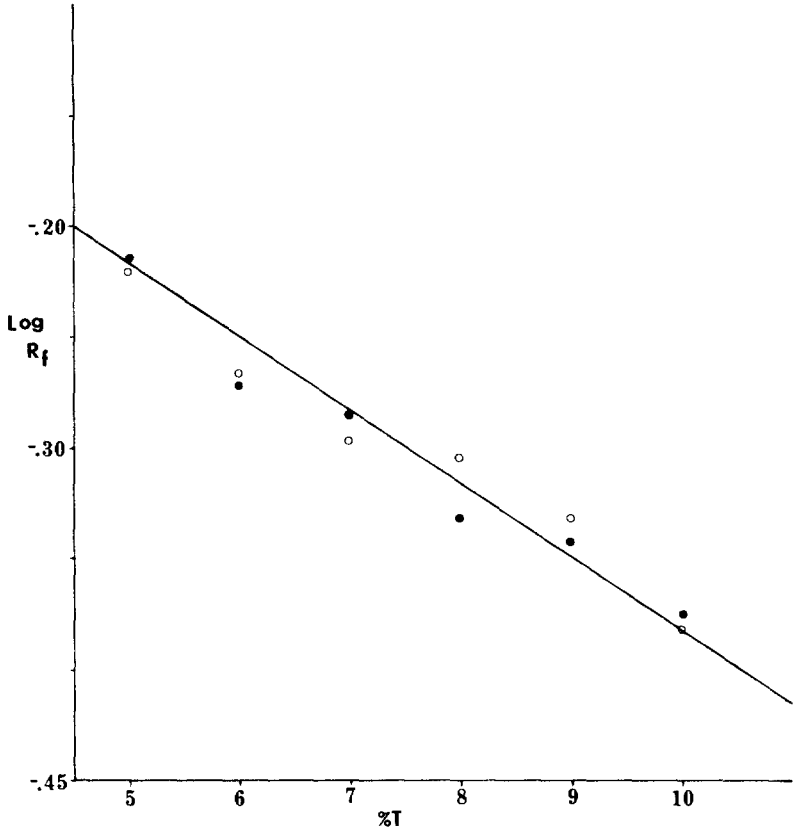

FIGURE 6.—Ferguson plot of (○) *F2*, $r^{3\text{-}55.4}$ and (●) *F2*, $R^{3\text{-}55.4}$. Points represent mean values (n = 3).

TABLE 6

Comparison of relative in vivo *and* in vitro *α-GPDH stability*

Strain	Rate of [^{3}H] incorporation[a]	*In vitro* stability[b]	Relative [*E*]	Relative *in vivo* stability[c]	Relative *in vitro* stability
F2, $R^{3\text{-}55.4}$	0.025 ± 0.002	0.064 ± 0.007	1.51	0.66	0.67
F2, $r^{3\text{-}55.4}$	0.021 ± 0.001	0.096 ± 0.012	1.00	1.00	1.00

[a] Values represent mean ± S.E.; n = 5.
[b] Values represent mean ± S.E.; n = 10.
[c] Calculated from the steady-state equation; $kd = ks/[E]$.

It has previously been reported that *in vitro* enzyme stability generally correlates with the proteins' relative *in vivo* degradation rate (*cf.* BOND 1975). We decided, therefore, to independently estimate the influence of the $R^{3\text{-}55.4}$ locus on *in vitro* α-GPDH stability. The results presented in Table 6 are in good agreement with the relative *kd* values estimated *in vivo* and thus corroborates the finding that the $R^{3\text{-}55.4}$ locus acts by affecting relative α-GPDH stability.

DISCUSSION

Although a good deal of work remains to be done on the $R^{3\text{-}55.4}$ system, some contrasts and similarities can presently be drawn between the properties of $R^{3\text{-}55.4}$ and those of other more or less well-characterized eukaryotic regulatory genes.

Nonproximal trans *regulatory control:* We have mapped (*3*-55.4) and characterized a *trans*-acting gene that exerts a regulatory effect on the spatial and temporal expression of α-GPDH—an enzyme whose structural locus maps to a separate chromosome (*2*-17.8). The existence of such distantly acting regulatory elements are well documented in a variety of eukaryotes including corn (LAI and SCANDALIOS 1980), mice (*cf.* PAIGEN 1979) and Drosophila (*e.g.* ABRAHAM and DOANE 1978; MCDONALD and AYALA 1978a,b; LAURIE-AHLBERG *et al.* (1980).

Dominance-recessive inheritance: Our gene was characterized by the presence of two alleles, one dominant ($R^{3\text{-}55.4}$) and one recessive ($r^{3\text{-}55.4}$) with regard to their regulatory effects on the level of α-GPDH in adult thoracic tissue. Dominance-recessive inheritance of allelic variation at distantly acting enzyme regulatory loci has been observed previously in higher organisms (RECHCIGL and HESTON 1967; GANSCHOW and SCHIMKE 1969; GANSCHOW and PAIGEN 1967; LALLEY and SHOWS 1977; DIZIK and ELLIOT 1977, 1978) including Drosophila (MCDONALD and AYALA 1978a,b; COCHRANE and RICHMOND 1979). In a number of instances, however, additive type inheritance has also been observed in both mice (*e.g.*, PAIGEN 1977; LUSIS and WEST 1978) and Drosophila (*e.g.*, DICKINSON 1972, 1975; ABRAHAM and DOANE 1978).

Specificity of the regulatory effect: We have found that the regulatory action of the $R^{3\text{-}55.4}$ locus is nonubiquitous in that its effect on α-GPDH is observed in thorax but not abdominal sections. In addition, the locus was found to have no significant influence on the levels of most soluble proteins including the abundant Drosophila enzymes ADH and PGI. The specificity of the regulatory effect of $R^{3\text{-}55.4}$ is incomplete, however, for the locus was found to modify the level of at least one other abdominal protein.

Tissue specificity of distantly acting regulatory effects have previously been observed in eukaryotes (*e.g.*, LAI and SCANDALIOS 1980; PAIGEN 1979) including Drosophila (ABRAHAM and DOANE 1978). In addition, a number of *trans*-acting regulatory genes have been purported to be target (protein) specific in their action (PAIGEN 1979). However, such claims can, at present, only be taken as tentative in that searches for effects on other gene products are typically limited.

Biochemical basis of the regulatory effect: We have found that the $R^{3\text{-}55.4}$ locus exerts its influence on α-GPDH by altering the enzyme's *in vivo* stability. Most dominantly-recessively inherited regulatory gene variation that has thus far been analyzed in eukaryotes is believed to act posttranslationally (see PAIGEN 1979 for review; also LAI and SCANDALIOS 1980; COCHRANE and RICHMOND 1979). In several instances the posttranslational effect has been shown to modify the target enzyme's *in vivo* stability (RECHCIGL and HESTON 1967; GAN-

SCHOW and SCHIMKE 1969; LAI and SCANDALIOS 1980). Ours is the first direct evidence for the existence of such a mechanism of regulation in Drosophila.

Mode of action of $R^{3\text{-}55.4}$*:* It is tempting, if somewhat premature, at this point to speculate on the molecular mode of action of the $R^{3\text{-}55.4}$ locus. It seems likely that $R^{3\text{-}55.4}$ codes for a product that either directly or indirectly modifies the structure of its target protein(s) and thereby influences the target's *in vivo* stability. If such a mode of action is, in fact, involved in controlling α-GPDH levels in our system, we have been unable to detect the structural modification via standard gel sieving techniques. Alternatively, one may postulate that $R^{3\text{-}55.4}$ codes for a protein-, tissue- or perhaps cell-specific protease which directly affects the stability of the regulated gene product. Arguments have been presented to support the existence of both mechanisms of control in eukaryotes (*cf.*, PAIGEN 1979; LAI and SCANDALIOS 1980). Experiments are currently underway in our laboratory to allow us to discriminate between these alternatives.

Overview: We have described a locus ($R^{3\text{-}55.4}$) that is inherited in a dominant-recessive fashion and is responsible for altering the *in vivo* stability of α-GPDH protein. Other *trans*-acting loci that influence the *in vivo* stability of eukaryotic proteins have been reported to exhibit dominant-recessive inheritance (*e.g.*, RECHCIGL and HESTON 1967; LAI and SCANDALIOS 1980), whereas *trans*-acting loci that influence the synthesis rate for specific proteins have been reported to be inherited in an additive fashion (*e.g.*, PAIGEN 1977; LUSIS and WEST 1978; ABRAHAM and DOANE 1978). It will be interesting to determine which modes of inheritance and molecular action characterize the great diversity of *trans*-acting genetic variation known to be segregating in natural populations of Drosophila. Such studies should help establish whether or not specific mechanisms of control are typically associated with enzymes of related function and/or patterns of tissue-specific expression and thus provide important insight into the overall question of eukaryotic gene expression.

This work was supported by National Science Foundation grant BSR-8200965 to J. M.

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