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Spontaneous mutations affecting glycerol-3-phosphate dehydrogenase enzyme activity in Drosophila melanogaster

Akihiko KOGA[1], Ko HARADA[2], Shinichi KUSAKABE[1]
and Terumi MUKAI[2]

[1]*Department of Biology, College of Arts and Sciences, Hiroshima University, Hiroshima 730*
[2]*Department of Biology, Faculty of Science, Kyushu University, Fukuoka 812, Japan*

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ABSTRACT

Significant genetic variance in glycerol-3-phosphate dehydrogenase (GPDH) activity was observed between chromosome lines of *Drosophila melanogaster* that had each accumulated spontaneous mutations for approximately 300 generations. No restriction map variation was found in a 26-kb region surrounding the entire *Gpdh* gene. The restriction analysis used is capable of detecting insertions/deletions larger than 0.05 kb. The survey would also detect chromosomal recombinations that include the entire *Gpdh* coding region. Therefore, if the spontaneous mutations that affected the enzyme activity are located inside the *Gpdh* gene region, then they are base pair substitutions or structural changes that are smaller than the limit in resolution described above.

1. INTRODUCTION

Great variation of enzyme activity exists in natural populations of Drosophila (McDonald and Ayala, 1978; Laurie-Ahlberg et al., 1980; Maroni and Laurie-Ahlberg, 1983; Yamazaki and Matsuo, 1984). To understand the mechanisms by which variation is maintained, it is beneficial to study the composition of spontaneous mutations which affect enzyme activity. Tachida et al. (1989) examined the restriction map variation in a 14-kb *Amy* gene region among chromosome lines that showed a significant variation in amylase enzyme activity. They found that the amylase activity variation was caused largely by a genetic change that had occurred in the region of the *Amy* structural gene. The genetic change was the replacement of a DNA fragment by the corresponding region of the homologous chromosome. Genetic variation was also observed in ADH (alcohol dehydrogenase) activity by Mukai, Harada and Yoshimaru (1984). Aquadro et al. (1990) confirmed the increased ADH activity variation, and conducted a molecular analysis of a 13-kb *Adh* gene region. Unlike the results of Tachida et al. (1989), no restriction map variation was found. Their restriction mapping analysis showed that the mobilization of transposable elements had not occurred in the *Adh* gene region in the chromosome lines used. The results also argue against

the possibility of gene exchange like that observed in the *Amy* gene region.

We conducted measurements of GPDH (glycerol-3-phosphate dehydrogenase) activity using samples from the same chromosome set as that of Aquadro et al. (1990). A significant increase in the variance in the enzyme activity was found. In *Drosophila melanogaster*, the *Gpdh* gene is located on the second chromosome [26A1-2 (Cook et al., 1986)] along with the *Adh* [35A4-B1 (Chia et al., 1985)] and the *Amy* [54A1-B1 (Gemmil, Levy and Doane, 1985)] genes. Two experimental tools were available to carry out a more precise molecular analysis in the *Gpdh* gene region than was possible in the *Adh* gene region. One is a chromosome strain whose second chromosome carries a deficiency that includes the entire *Gpdh* gene and its flanking sequences. This deficiency enabled us to make restriction maps of single chromosomes. The other is three base pair differences that segregate between the *Cy* and the *l* chromosomes (two homologous chromosomes balanced in the mutation accumulation system). Our restriction mapping showed that the increased variation in GPDH activity was not caused by large structural changes in the *Gpdh* gene region.

2. MATERIALS AND METHODS

Accumulation of spontaneous mutations

The mutation accumulation chromosome lines (known as JH lines) were de-

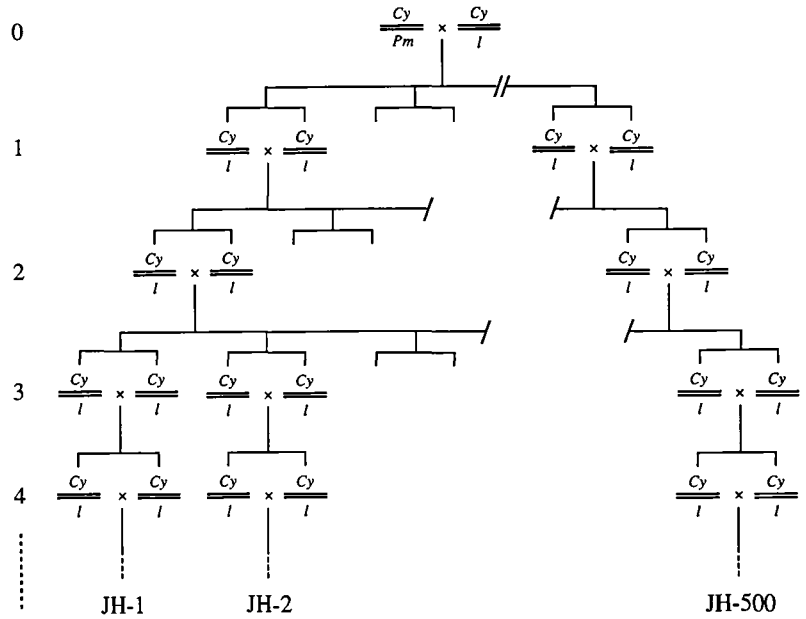

Fig. 1. Mating scheme for the accumulation of spontaneous mutations. The generation number is shown on the left side. *Cy* and *Pm* stand for multiple inversions *In(2LR)SM1* and *In(2LR)bw*^{V1}, respectively.

veloped by T. Mukai, H. E. Schaffer and C. C. Laurie (cf. Mukai and Cockerham, 1977). The mutation accumulation procedure is schematically shown in Fig. 1 and is outlined below. An *In(2LR)SM1* balancer chromosome (*Cy*) and a standard chromosome (*l*), both carrying recessive lethals at different loci, were amplified within three generations by a marked inversion technique with a balancer strain C160 (*Cy / Pm*). Five hundred chromosome lines (JH-1, JH-2, ..., JH-500), which were each heterozygous for the second chromosomes *Cy* and *l*, were established at the third generation, and were maintained by single-pair matings or five-pair matings for about 300 generations. Each chromosome line accumulated spontaneous mutations under very little pressure of natural selection during this period. From the 500 JH lines, 26 chromosome lines were randomly chosen, and the genetic backgrounds (chromosomes *1*, *3* and *4* and cytoplasm) were replaced by that of strain C160 through repeated backcrossing. Concurrent with the last generations of the backcrosses, a set of control chromosome lines was prepared by amplifying one of the JH lines in two generations by the marked inversion technique with C160.

Measurement of GPDH activity

The following procedures were applied to each of the 26 JH and the 26 control lines. Twenty *Cy/l* females and 20 *Cy/l* males were collected, and crosses with 5 females and 5 males were made in 4 different vials. Two of the four vials were used in the first set of activity assays, and the remaining two in another set. From each vial, 4-day old F_1 males of *Cy/l* were collected, and 5 of these were homogenized in 200 μl of distilled water. After centrifugation, 25 μl of supernatant was mixed with 675 μl of glycine-NaOH buffer (pH9.5) containing 16 mM α-glycerophosphate and 4.5 mM NAD^+. Change in the absorbance at a wave length of 340 nm was measured. The activity in units per 700 μl of the sample solution was calculated as the regression in 1 minute multiplied by 0.1125 [$=700/(6.22\times10^3)$], where 700 is the sample volume and 6.22×10^3 is the extinction coefficient of NADH.

The hierarchical structure of our data is: 2 groups (JH lines and the control lines)$\times$2 sets$\times$26 lines$\times$2 replications. Each set of measurements was conducted in a single day. In each set, the groups (JH lines or the control lines) were ignored, and the sequence of the measurements was randomized among a total of 52 lines (26 JH lines and 26 control lines).

Restriction map analysis

Restriction mapping by the Southern-hybridization technique was applied to the JH lines. In each of the 26 JH lines, males of *Cy*/l** were crossed with *Cy/Df* females. The asterisk represents chromosomes that experienced the mutation accumulation (only in this section). *Df* stands for the second chromosome carrying a deficiency which covers the entire *Gpdh* gene [*Df(2L)GdhA* (Kotarski et

al., 1983)] and a dominant marker *L(Lobe)* which causes reduction in eye size. In the next generation, about 100 *Cy*/Df* and *l*/Df* adult flies were collected and frozen in separate tubes at −70°C. Genomic DNA was extracted with a method outlined by Bingham, Levis and Rubin (1981). In each of the *Cy*/Df* and *l*/Df* samples, the genomic DNA was partitioned into four tubes and was digested completely with different enzymes: *Bam*HI, *Eco*RI, *Hin*dIII, and *Sac*I, which are all hexanucleotide-specific restriction enzymes. Electrophoresis through 0.9% agarose gels, blotting to nylon membranes, and hybridization with ^{32}P-labelled probes were carried out (cf. Southern, 1975; Sambrook, Fritsch and Maniatis, 1989; Feinberg and Vogelstein, 1983). The probes used were pG8*Sl* and pG9*E* (Takano et al., 1989) which cover the 26-kb region shown in Fig. 2. The region includes the maximum *Gpdh* transcriptional unit of 5.5 kb, an upstream region of 11.0 kb and a downstream region of 9.5 kb (cf. Kusakabe et al., 1991). The resulting restriction pattern was analyzed for any change in the mobility of restriction fragments.

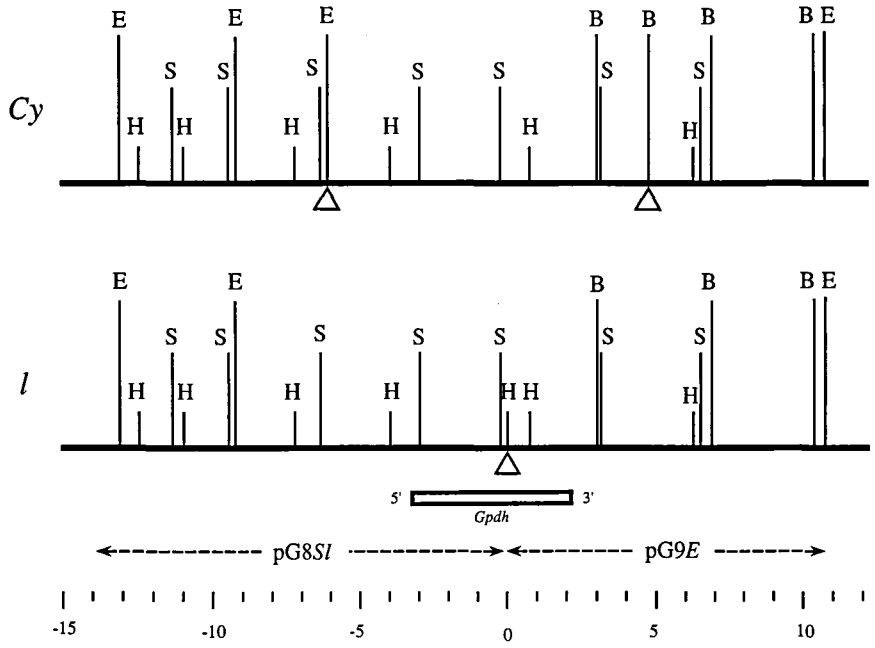

Fig. 2. Restriction maps of the 26-kb *Gpdh* gene region of the *Cy* (upper) and the *l* (lower) chromosomes. The symbols for restriction sites are: B=*Bam*HI, E=*Eco*RI, H=*Hin*dIII and S=*Sac*I. The maximum *Gpdh* transcriptional unit is shown by a box. Triangles under the maps indicate restriction sites that are unique to one of the two chromosomes. The dotted arrows show the regions covered by the probes.

3. RESULTS AND DISCUSSION

Two-way analysis of variance of the activity data was made separately for the JH and the control lines. The results are given in Table 1. In the analysis, both factors, sets and lines, were taken as random factors. The difference between lines was significant at the 0.1% level in the JH lines, while no significant difference was detected in the control lines. The significant difference between the JH lines was apparently caused by spontaneous mutations that had accumulated on the second chromosomes during the 300 generations. The genetic variance component of lines with its standard deviation is estimated to be 1.6818 ± 0.6237 ($\times 10^{-7}$ units^2) for the JH lines, and 0.1558 ± 0.2470 ($\times 10^{-7}$ units^2) for the control lines. The difference between sets was significant at the 0.1% level in both the JH and the control lines, but the interaction between sets and lines was not significant in either group. The latter indicates that the ranking of GPDH activity among chromosome lines did not change significantly on different days (sets).

Table 1. Analysis of variance of GPDH activity

Source	Sum of squares ($\times 10^5$)	d. f.	Mean square ($\times 10^5$)	F
(1) JH lines				
Sets	0.6441	1	0.6441	30.84***
Lines	2.2041	25	0.0882	4.22***
Interaction	0.5222	25	0.0209	0.84^{NS}
Error	1.2919	52	0.0248	
Total	4.6622	103		
(2) Control lines				
Sets	0.4536	1	0.4536	15.80***
Lines	0.8732	25	0.0349	1.22^{NS}
Interaction	0.7176	25	0.0287	0.65^{NS}
Error	2.2853	52	0.0440	
Total	4.3296	103		

*** Significant at 0.1% level.
NS Not significant ($P > 0.05$).

The restriction pattern of the 26-kb *Gpdh* gene region was examined using four different restriction enzymes for each of the *Cy* and the *l* chromosomes from every JH line. The restriction pattern obtained was identical among 26 *Cy* chromosomes and among 26 *l* chromosomes. Fig. 2 presents the restriction maps of the region. The two maps are different from each other at three positions: the *Eco*RI site at position −6.2 (2.7 kb upstream from the 5′ end of the *Gpdh* coding

region), *Hin*dIII at 0.0 (near the center of the coding region), and *Bam*HI at 4.9 (2.5 kb downstream from the 3′ end of the coding region). Comparing the restriction maps with the sequence information of Bewley et al. (1989) revealed that the *Hin*dIII site at 0.0 is located in the 4th intron.

As described above, a significant increase in the genetic variation of GPDH activity was observed among chromosomes of the same origin. On the other hand, no restriction map variation was detected in the 26-kb region encompassing the entire *Gpdh* structural gene. In our studies, Southern blot analysis has the highest resolution for restriction fragments in the range of 2–4 kb. Differences larger than 0.05 kb in the mobility of restriction fragments can be empirically detected in this range. Any points in the 26-kb region surveyed are included in one or more restriction fragments of these sizes (see Fig. 2). Therefore, when the genetic changes that caused the increased variation of GPDH activity are assumed to lie in the *Gpdh* gene region, the following three models are possible: (1) insertions or deletions of sizes less than 0.05 kb, (2) base pair substitutions, and (3) replacements of DNA fragments.

The first model is difficult to support because there are no known transposable element families in Drosophila whose sizes are less than 0.05 kb (cf. Finnegan and Fawcett, 1986). The second explanation may be possible. This possibility had been examined extensively by Aquadro et al. (1990) using quantitative data of *Adh* restriction map variation. When their logic and estimates are applied to our data, 1 to 2 base pairs are expected to have changed in the total region examined (52 chromosomes$\times$26,000 base pairs). It is possible that a single base pair change has an effect on gene expression. The last model is possible only for replacement of small DNA fragments (Its range is specified below.). Tachida et al. (1990) found that replacement of an *Amy* gene region of the *Cy* chromosome by that of the *l* chromosome contributes significantly to the increase in amylase activity variation. They note the possibility of gene conversion and double crossing over as the mechanisms by which the replacements occur. In the present molecular analysis of the *Gpdh* gene region, there are three segregating sites at positions -6.2, 0.0 and 4.9 (see Fig. 2). Gene exchanges that include at least one of these three sites had not occurred. We cannot rule out the possibility of gene exchanges that do not include any of these three sites. In fact, it was reported that gene conversion of these sizes is common in the *rosy* gene region (Curtis et al., 1989).

The three models discussed above were made on the assumption that genetic changes located in the *Gpdh* gene region are responsible for the increased GPDH activity variation. Another explanation is possible, *i.e.*, the genetic changes that are located outside the *Gpdh* gene region are the predominant cause of the increased GPDH activity variation.

It is of interest to see if ADH and GPDH share some genetic factors which control enzyme activity. Sets of activities of ADH and GPDH are plotted in Fig.

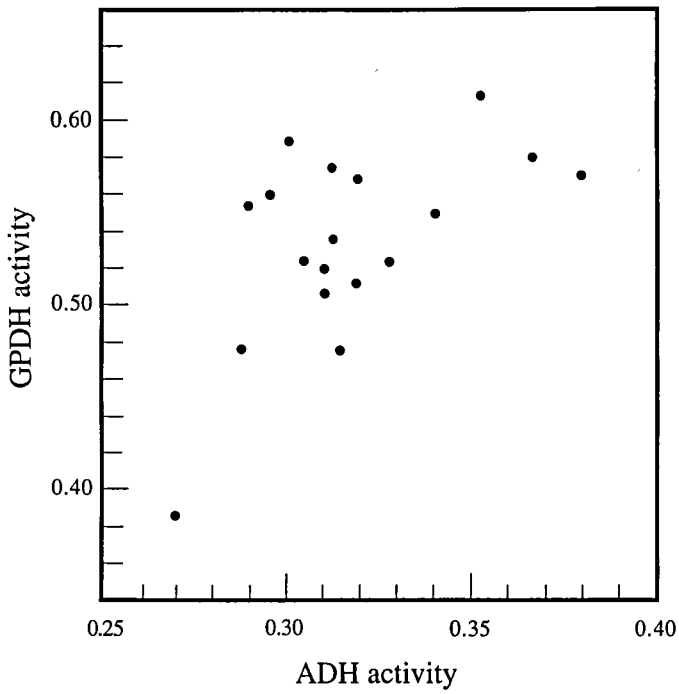

Fig. 3. Relationship between the ADH and the GPDH activities. Data for the ADH activities are from Aquadro et al. (1990).

3. Eighteen chromosome lines were available for this analysis. The correlation coefficient was calculated to be $r=0.59$. This value is significantly different from 0 ($t=2.90$, d.f.$=16$, $0.01<P<0.02$). However, it is obvious from Fig. 3 that a single line in the lower left hand corner contributes significantly to the positive correlation. When this line is excluded, the correlation coefficient is $r=0.44$, and the statistical significance disappears ($t=1.92$, d.f.$=15$, $0.05<P<0.10$). Therefore, conclusions cannot be made at present. The long-term mutation accumulation lines are a unique resource for this kind of analysis.

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