UC Irvine

UC Irvine Previously Published Works

Title

Isolation and characterization of the glucose-6-phosphate dehydrogenase gene of *Drosophila melanogaster*.

Permalink

<https://escholarship.org/uc/item/8r8221bk>

Journal

Gene, 35(1-2)

ISSN

0378-1119

Authors

Ganguly, R
Ganguly, N
Manning, JE

Publication Date

1985

DOI

10.1016/0378-1119(85)90161-1

Copyright Information

This work is made available under the terms of a Creative Commons Attribution License, available at <https://creativecommons.org/licenses/by/4.0/>

Peer reviewed

GENE 1270

Isolation and characterization of the glucose-6-phosphate dehydrogenase gene of *Drosophila melanogaster*

(Immunoselection of mRNA; library screening; in situ hybridization; gene copy number; dosage compensation; λ vector, recombinant DNA; cloning)

Ranjan Ganguly, Nivedita Ganguly and Jerry E. Manning*

Department of Molecular Biology and Biochemistry, School of Biological Science, University of California at Irvine, Irvine, CA 92717, (U.S.A.) Tel. (714)856-7102

(Received October 9th, 1984)

(Revision received December 27th, 1984)

(Accepted January 8th, 1985)

SUMMARY

To investigate the molecular basis of dosage compensation in *Drosophila*, a recombinant λ phage containing the *Drosophila melanogaster* glucose-6-phosphatase dehydrogenase (G6PD) gene was isolated by differential screening of a *Drosophila* genomic λ library with poly(A)⁺ RNA obtained from polyribosomes enriched for or depleted of G6PD mRNA sequences. Of 44 000 plaques screened, a single phage, λ DmG21, showed hybridization with the enriched poly(A)⁺ RNA but not the depleted one. Confirmation that the *Drosophila* DNA fragment cloned in λ DmG21 contains the G6PD gene sequence is based on the following observations. λ DmG21 DNA shows hybridization only to the 18D region of the salivary gland X-chromosome, which is the known cytological locus for the G6PD gene. In vitro translation of the poly(A)⁺ mRNA selected by hybridization to λ DmG21 DNA sequences shows a polypeptide product of apparent M_r 55 000, identical to that of the monomeric unit of G6PD. When the putative coding sequence of G6PD is cloned into the expression vector λ gt11, recombinant plaques are recognized by anti-G6PD immunoglobulin. A transcriptional map of the G6PD gene shows that it is divided into two exons, 0.9 kb (exon I) and 1.8 kb (exon II) long, which are separated by a 2.4-kb intron. The G6PD mRNA is 2.0 kb in length and the steady-state level of the mRNA is similar in both sexes. Measurement of the copy number of the G6PD gene in males and females shows the gene to be present once per X-chromosome in both sexes. No amplification of the gene sequence was observed in males. These results are, therefore, in agreement with the previous suggestion that dosage compensation is the result of enhanced transcription of X-linked genes in males.

* To whom correspondence and reprint requests should be addressed.

Abbreviations: Adh, alcohol dehydrogenase; bp, base pairs; DBM, diazobenzyloxymethyl; G6PD, glucose-6-phosphate de-

hydrogenase; IgG, immunoglobulin; kb, kilobases or 1000 bp; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; R_{6t} , mol of ribonucleotides per liter $\times$ time (s); SDS, sodium dodecyl sulfate; 6PGD, 6-phosphogluconate dehydrogenase; TCA, trichloroacetic acid; TEMG, see MATERIALS AND METHODS, section a; u, units.

INTRODUCTION

Glucose-6-phosphate dehydrogenase (G6PD; EC 1.1.1.49) is widely distributed in the animal kingdom and catalyzes the first reaction in the pentose phosphate pathway. In *D. melanogaster*, the gene encoding G6PD has been genetically mapped to the X-chromosome at position 1–63.6 and is designated Zw (Young et al., 1963). Cytogenetic analysis has further localized Zw within the cytological interval proximal to 18D1-2 but distal to 18F (Stewart and Merriam, 1974).

G6PD is one of a small number of X-linked enzymes that have been extensively utilized for investigation of dosage compensation at the level of enzyme synthesis (for review, see Lucchesi, 1977; Stewart and Merriam, 1980). Dosage compensation is the equalization of X-linked gene products in males and females (Muller et al., 1931). Unlike mammals, where equalization of X-linked gene products occurs by X-chromosomal inactivation (Lyon, 1961) both X-chromosomes remain active in female *Drosophila* (Kazazian et al., 1965). The equalization of X-linked gene products in male *Drosophila*, which have one dose of X-linked genes and female *Drosophila*, which have two doses of such genes, is believed to be mediated by enhancing the level of transcription of X-linked genes in males (Mukherjee and Beerman, 1965; Korge 1970; Holmquist, 1972). Although these studies suggest that the steady-state level of mRNA transcripts from dosage compensated X-linked genes would be equivalent in males and females, no direct evidence has been published.

Recent studies have shown that the autosomal-linked xanthine dehydrogenase gene (Spradling and Rubin, 1983) and dopadecarboxylase gene (Scholnick et al., 1983) show partial dosage compensation when inserted into the X-chromosome via P-element-mediated transformation (Spradling and Rubin, 1982; Rubin and Spradling, 1982). Conversely, seven cases have been observed in which the X-linked white gene was inserted into autosomal sites, and in each case the white gene was partially dosage-compensated (Hazelrigg et al., 1984). These observations have led to the suggestion that *cis*-acting 'receptor' sequences may mediate dosage compensation and that such sequences are numerous on the X-chromosome (Hazelrigg et al., 1984).

This hypothesis suggests that other X-linked genes

may have closely adjacent receptor sequences and thus retain the ability to exhibit dosage compensation when translocated to autosomal sites. To determine if this is the case, and to investigate other possible mechanisms that might mediate dosage compensation in *Drosophila*, we have chosen the gene encoding G6PD as a model. In this paper we report the isolation and structural organization of the G6PD gene sequence in *D. melanogaster*. We also present evidence that dosage compensation of the G6PD gene in males is manifested at the level of transcription.

MATERIALS AND METHODS

(a) Purification of glucose-6-phosphate dehydrogenase and preparation of anti-G6PD immunoglobulin

Adult *D. melanogaster* (Oregon R⁺; 10 g) was homogenized at 4°C in 80 ml of 50 mM Tris, pH 8.0, 1 mM EDTA, 7 mM 2-mercaptoethanol, 10% glycerol (TEMG; Steele et al., 1969). The homogenate was centrifuged at 16000 × *g* for 30 min, and the supernatant was applied to a 2', 5'-ADP agarose (PL Biochemicals) affinity column (110 × 10 mm) equilibrated with TEMG. After washing with 45 ml of TEMG, G6PD was eluted with 9.0 ml of TEMG containing 0.1 mM NADP⁺ (Brodelius et al., 1974). The enzyme was placed on a DE-52 cellulose column (50 × 5 mm) equilibrated with TEMG and the column was washed with 10 ml of TEMG, containing 50 mM NaCl. The enzyme was eluted with 5 ml TEMG containing 150 mM NaCl. In a typical experiment, the yield of G6PD was determined to be 24%, and the specific activity was approx. 70 u/mg protein. The purification factor was 1296, in excellent agreement with previously reported values (Hori and Tanda, 1980). Enzyme activity at each step of purification was assayed according to Steele et al. (1969).

SDS-PAGE of the purified enzyme showed a major Coomassie blue-stained band of apparent *M_r* 55000, which is the apparent *M_r* of the monomeric unit of G6PD (Lee et al., 1978). A minor band, representing less than 10% of the intensity of the 55-kDal band was observed at apparent *M_r* 65000. The

enzyme preparation was free of contamination by 6PGD activity, as assayed by staining the gel with 6PGD specific stain (Hori and Tanda, 1980).

Rabbit antibody against G6PD was prepared as described by DeFlora et al. (1977). Anti-G6PD IgG was purified from rabbit serum by $(\text{NH}_4)_2\text{SO}_4$ precipitation, DE-52 cellulose column chromatography (Weir, 1967), and affinity chromatography on a protein A-Sepharose CL-4B column (Goudswaard et al., 1978). Heparin (20 u/ml) was added to the antibody preparation to inhibit RNase activity.

(b) Enrichment of G6PD mRNA

Polyribosomes were isolated from adult *Drosophila* by the method of Benyajati et al. (1980), and suspended in polyribosome buffer (0.3 M NaCl, 50 mM Tris, pH 7.5, 5 mM MgCl_2 , 1 $\mu\text{g}/\text{ml}$ of cycloheximide, 20 u/ml of heparin) at a concentration of 1000 A_{260} u/ml. One ml of anti-G6PD IgG, at 1 mg/ml in PBS was added to 1.0 ml of polyribosomes, and the mixture was incubated for 2 h at 4°C. The mixture was applied to a 1.5-ml protein A-Sepharose CL-4B (Pharmacia) column at 4°C and washed with 80 ml of polyribosome buffer; mRNA in bound polyribosomes was eluted with 3.0 ml of 25 mM Tris, pH 7.6, 20 mM EDTA, 20 u/ml of heparin (Shapiro and Young, 1981). RNA in the unbound polyribosome fraction (i.e., depleted polyribosomes) and in the specifically bound polyribosomes (i.e., enriched polyribosomes) was further purified by phenol-chloroform extraction, and polyadenylated RNA was isolated by oligo(dT)-cellulose chromatography (Levy and Manning, 1981). This procedure was repeated until approx. 10000 A_{260} u of polyribosomes had been processed. Total poly(A)⁺ RNA obtained from the depleted and enriched polyribosomes were 2.0 mg and 14 μg , respectively.

(c) Determination of transcription orientation

(1) S1 protection assay

The 1.2-kb *Bgl*II-*Ava*I fragment (see Fig. 2) within exonII was ^{32}P -labeled at the 5' terminus and digested with endonuclease *Pst*I to generate a 0.4-kb *Bgl*II-*Pst*I fragment and 0.8-kb *Pst*I-*Ava*I fragment. These DNA fragments (approx. 1×10^{-3} μg of coding sequence) were hybridized to adult polyribo-

somal poly(A)⁺ RNA (30 μg) under conditions that allow RNA-DNA hybridization, but do not allow DNA-DNA reannealing (Casey and Davidson, 1977; Levy et al., 1982). The R_{0t} value achieved was 460, which is approx. ten times that required for saturation hybridization under these conditions (Levy et al., 1982). After hybridization the sample was treated with S1 nuclease and the RNA-protected DNA was electrophoresed on an alkaline agarose gel (Maniatis et al., 1982). After electrophoresis the gel was neutralized in 3 M NaCl, 0.5 M Tris, pH 7.0, blotted to nitrocellulose, and exposed for autoradiography.

(2) Southern hybridization

G6PD-enriched poly(A)⁺ RNA was partially hydrolyzed at alkaline pH and 5'-end labeled as described (Levy et al., 1982). End-labeled RNA was purified by Sephadex G-50 chromatography and fractionated by oligo(dT) chromatography into a 5'-enriched probe (unbound) and a 3'-enriched probe (bound). Restriction fragments spanning different parts of the G6PD gene (see RESULTS, section c) were electrophoresed on agarose gels, Southern transferred, and hybridized with 5'- or 3'-end-enriched RNA probes (Levy et al., 1982). The blots were subjected to autoradiography.

(d) Radiolabeling of DNA and RNA

DNA was nick-translated with [α - ^{32}P]dCTP (Amersham) to a specific activity of $2\text{--}4 \times 10^8$ cpm/ μg using a nick-translation kit from Bethesda Research Laboratory (BRL). The radiolabeled DNA was then purified as described by Levy et al. (1982). RNA was 5'-end-labeled to a specific activity of $3\text{--}5 \times 10^7$ cpm/ μg using T4 polynucleotide kinase (Boehringer-Mannheim) and [γ - ^{32}P]ATP (Levy et al., 1982).

(e) Restriction enzymes, preparation of RNA and DNA, electrophoresis, Southern transfer, Northern transfer, library screening and subcloning

All restriction enzymes were purchased from BRL and used as recommended. Isolation and electrophoresis of nucleic acids and Southern and Northern transfers are described elsewhere (Levy and Manning, 1981; Levy et al., 1982). Hybridization of

5'-end-labeled RNA to Southern blots and filters for library screening was according to Fouts et al. (1981). Northern blots were prehybridized and hybridized as described by Thomas (1980).

The DNA clone described here was selected by in situ plaque hybridization technique (Benton and Davis, 1977) from a library of *D. melanogaster* genomic DNA cloned in phage λ Charon 4 (Maniatis et al., 1978).

The DNA fragments, 3.0-kb *SalI* and 3.9-kb *EcoRI*, containing exon I and exon II, respectively, were subcloned in the polylinker of the vector pUC9 (Vieira and Messing, 1982), and the subclones were named pDmG20/3.0*SalI* and pDmG20/3.9RI, respectively. A subclone, sAC-1 (Goldberg, 1980), containing the *Adh* gene of *D. melanogaster* was obtained from Dr. J.C. Lucchesi, University of North Carolina.

(f) Hybrid selection of G6PD mRNA

For hybrid selection of G6PD mRNA, recombinant plasmid DNA pDmG20/3.9RI containing exon II was linearized with *HindIII* and covalently coupled to DBM-cellulose (Anderson et al., 1979). Enriched polyribosomal poly(A)⁺ RNA (1 μ g) was hybridized to 1.3 μ g of cellulose-bound DNA in 10 μ l of 70% formamide containing 0.1 M Tris, pH 7.8, 0.3 M NaCl, 10 mM EDTA at 46°C for 24 h. The DNA-DBM-cellulose was washed three times with 200 μ l of 0.3 M Na⁺ acetate, pH 7.5, three times with 200 μ l of 55% formamide containing 0.7 M NaCl, 0.2 M Tris, pH 7.5, at 25°C and twice with 200 μ l of the 55% formamide solution at 42°C. Hybridized RNA was recovered by incubating the DNA-DBM-cellulose in 300 μ l of 99% formamide for 2 min at 60°C followed by ethanol precipitation (Anderson et al., 1979). The hybrid-selected poly(A)⁺ RNA was translated in a rabbit reticulocyte lysate system (BRL), and products were analyzed by SDS-urea PAGE.

RESULTS

(a) Isolation of the G6PD gene

An electrophoretic profile of the translation products of enriched and depleted poly(A)⁺ RNA is

Fig. 1. Identification of G6PD mRNA in polyribosomal poly(A)⁺ RNA enriched for and depleted of G6PD mRNA. Enriched or depleted poly(A)⁺ RNA (approx. 1 μ g) was translated in a rabbit reticulocyte cell-free translation system (Bethesda Research Laboratory) using [³H]leucine as label. To analyze G6PD synthesis 20 μ l of post-ribosomal supernatant of the reaction mixture (approx. 25000 TCA-precipitable cpm) 30 μ l of PBS, 2.5 μ g anti-G6PD-IgG and 30 μ g goat anti-rabbit-IgG (Sigma), were mixed and immunoprecipitated (Rosen, 1976). The precipitate was dissolved in 20 μ l sample buffer (Laemmli, 1970), heated at 100°C for 2 min and adjusted to 5 M urea. For total product analysis 10 μ l of the post-ribosomal supernatant was digested with 100 μ g/ml RNase for 30 min at 37°C and adjusted with sample buffer and urea. The total (lanes a, c) and anti-G6PD-IgG-precipitable products (lanes b, d) synthesized in the cell-free system were analyzed by SDS-urea PAGE (Storti et al., 1976) and fluorography. Lanes a, b: enriched poly(A)⁺ RNA; lanes c, d: depleted poly(A)⁺ RNA; lane e shows polypeptide synthesized by globin mRNA which is not precipitated by anti-G6PD-IgG (lane f). The arrow shows a 55-kDal polypeptide synthesized by the enriched poly(A)⁺ RNA. *M_r* markers (left margin): BSA (66 kDal), ovalbumin (45 kDal), pepsin (34.7 kDal), trypsinogen (24 kDal).

shown in Fig. 1. Although the major translation products of enriched and depleted poly(A)⁺ RNA of the two RNAs are essentially identical, a minor polypeptide of apparent *M_r* 55 000, the size of the G6PD monomer (Lee et al., 1978), is visible only in the translation products directed by the enriched poly(A)⁺ RNA. Immunoprecipitation of these pro-

Fig. 3. Chromosomal localization of the G6PD gene. λ Dmg21 was labeled with $[^3\text{H}]\text{dCTP}$ and $[^3\text{H}]\text{dTTP}$ by nick-translation and hybridized to the salivary gland polytene chromosomes (Levy et al., 1982). The results show that the DNA hybridizes to the 18D region of the X-chromosome. Similar results were obtained when a 3.9-kb *Eco*RI fragment was used as a probe. The cytological map described by Lefevre (1976) was used for chromosomal localization.

(b) Measurement of G6PD mRNA size and relative abundance in males and females

$[^3\text{H}]$ Uridine incorporation into the single polytenic X chromosome of male larval salivary glands and the paired X chromosomes of females is equivalent (Mukherjee and Beermann, 1965), suggesting that dosage compensation is a transcriptional phenomenon. Since this observation suggests that the steady-state level of mRNA encoded by X-linked dosage compensated gene sequences is equivalent in males and females, we determined the relative level of G6PD mRNA in the two sexes.

Initially, the size and relative abundance of G6PD mRNA in the enriched and depleted poly(A)⁺ RNA

Fig. 4. In vitro translation of hybrid-selected G6PD mRNA. DNA of subclone pDmG20/3.9RI containing exonII was covalently coupled to diazobenzylloxymethyl cellulose and hybridized with enriched poly(A)⁺ RNA. Hybridized RNA was eluted and translated in a rabbit reticulocyte lysate, and the products were subjected to SDS-urea PAGE (lane b). Products synthesized in the absence of exogenous RNA are shown in lane a. The arrow denotes the position of the 55-kDa polypeptide in lane b. M_r markers as in Fig. 1.

preparations was measured by Northern blot hybridization. As shown in Fig. 5, the length of the G6PD mRNA transcript is 2.0 kb and the abundance of G6PD mRNA in the enriched poly(A)⁺ RNA is greater than in the depleted poly(A)⁺ RNA. Overexposure of the autoradiogram of Fig. 5 showed the presence of a minor band of length 2.3 kb. Possible implications of this RNA species are discussed below (see DISCUSSION, section b). To quantitate the relative abundance of the two RNA fractions, the area of each blot containing only the intensely hybridizing band was removed and counted by liquid scintillation. When the 3.9-kb *Eco*RI fragment was used as the probe, G6PD mRNA transcripts were seen to be 67 times more

Fig. 5. Blot hybridization of poly(A)⁺ RNAs enriched for and depleted of G6PD mRNA sequences. Northern blots of poly(A)⁺ RNA enriched for or depleted of G6PD mRNA sequences were hybridized either with [³²P] DNA of subclone pDmG20/3.9RI (lanes a–c) or pDmG20/3.0 *Sal*I (lanes d–f). Lanes a, b: 0.5 μ g of enriched poly(A)⁺ RNA from polyribosome preparations 1 and 2, respectively; lane c: 10.0 μ g of depleted poly(A)⁺ RNA from preparation 1; lane d: 0.2 μ g of enriched poly(A)⁺ RNA from preparation 2; lanes e, f: 0.2 and 5.0 μ g, respectively, of depleted poly(A)⁺ RNA from preparation 1. Numbers in kb on the margin refer to the migration of ³²P-labeled *Hind*III fragments of phage λ DNA.

abundant in the enriched poly(A)⁺ RNA than in the depleted poly(A)⁺ RNA (Fig. 5, lanes a–c). A similar difference (i.e., 58-fold) in the abundance of G6PD mRNA transcripts between the two RNAs was observed when the 3.0-kb *Sal*I fragment was used as the hybridization probe (Fig. 5, lanes d and f).

To determine the steady-state level of G6PD mRNA transcripts in male and female *Drosophila* equivalent amounts of polyribosomal poly(A)⁺ RNA from adult male and female flies were analyzed by Northern blot hybridization (Fig. 6). Quantitation of radioactivity in the hybridizing bands showed the

Fig. 6. Determination of the relative steady-state level of the G6PD mRNA level in male and female polyribosomal poly(A)⁺ RNA. Northern blots of glyoxalated RNA were hybridized with ³²P-labeled G6PD DNA (lanes a–d) or *Drosophila* Adh DNA (lanes e, f) and autoradiographed. Lanes a–d were exposed for six days, and lanes e and f were exposed for one day. Lane a: 1 μ g male RNA; lane b: 2 μ g male RNA; lane c: 1 μ g female RNA; lane d: 2 μ g female RNA; lane e: 0.5 μ g male RNA; lane f: 0.5 μ g female RNA. The areas of the blot containing the hybridizing band were excised and the radioactivity was determined. The results are shown in Table I. Size markers on left margin as in Fig. 5.

level of G6PD mRNA transcripts in adult male RNA to be slightly greater than that of the female RNA. Essentially identical results were obtained when the 3.0-kb *Sal*I fragment was used as the DNA probe (not shown). To determine whether the observed difference in the steady-state level of G6PD mRNA in males and females is a result of inaccuracy in RNA quantitation, the steady-state level of Adh mRNA in these two RNA samples was measured. As shown in Fig. 6 and Table I, male RNA has a slightly higher level of Adh mRNA than female RNA. The ratio of the steady-state levels of G6PD

TABLE I

Levels of G6PD and Adh mRNAs in male and female polyribosomal poly(A)⁺ RNA

Probes ^a	μg RNA	cpm hybridized ^b		G6PD/Adh ratio	
		Male	Female	Male	Female
G6PD DNA	1.0	581	454	0.13	0.11
G6PD DNA	2.0	1225	1146	0.27	0.29
Adh DNA	0.5	4555	3976	—	—

^a Respective areas of the Northern blot showing hybridization in Fig. 6 were excised out and radioactivity determined. Nitrocellulose of an equivalent area was excised where no hybridization was observed and counted to determine background radioactivity.

^b Probes used were ³²P-labeled pDmG20/3.9RI (G6PD DNA) and sAc-1 (Adh DNA).

mRNA and Adh mRNA in the two sexes is found to be essentially identical, thus indicating that the steady-state level of G6PD mRNA in male and female *Drosophila* is equivalent.

(c) Transcription orientation

Two methods were used to determine the transcriptional orientation of the G6PD gene on the restriction map shown in Fig. 2. First, a 0.4-kb *Bgl*II-*Pst*I fragment and a 0.8-kb *Pst*I-*Ava*I fragment were 5'-end-labeled at the *Bgl*II and *Ava*I sites, respectively, and hybridized with a large sequence excess of adult polyribosomal poly(A)⁺ RNA. Following S1 nuclease digestion (Fig. 7) only the 0.8-kb *Pst*I-*Ava*I fragment is protected, thus providing the 5'–3' orientation shown in Fig. 2.

In the second method enriched poly(A)⁺ RNA was partially hydrolyzed, 5'-end-labeled with [³²P]ATP (Levy et al., 1982), and chromatographed on an oligo(dT) cellulose column. The 5'-end-enriched (unbound) and 3'-end-enriched (bound) sequences were hybridized to Southern blots containing the following DNA fragments in equimolar amounts (Fig. 2): (a) 1.5-kb *Sal*I-*Bam*HI containing exon I; (b) 1.8-kb *Eco*RI-*Ava*I containing exon II and (c) 0.45-kb *Ava*I located at the 3' end of exon II. The 5'-end-enriched RNA sequences hybridized with the 1.5-kb *Sal*I-*Bam*HI and 1.8-kb

Fig. 7. Transcriptional orientation of the G6PD gene determined by S1 protection. The 0.4-kb *Bgl*II-*Pst*I and 0.8-kb *Pst*I-*Ava*I fragments in exon II were 5'-end labeled at the *Bgl*II and *Ava*I sites, respectively, hybridized to adult polyribosomal poly(A)⁺ RNA, digested with S1 nuclease, and autoradiographed following alkaline agarose gel electrophoresis (lane a). The electrophoretic profile of the ³²P-labeled fragments prior to hybridization is shown in lane b. Size markers on left margin as in Fig. 5.

*Eco*RI-*Ava*I fragments but not with the 0.45-kb *Ava*I fragment (results not shown). When 3'-end-enriched RNA sequences were used as a probe, hybridization was seen only with the 1.8-kb *Eco*RI-*Ava*I and 0.45-kb *Ava*I fragments, but not with the 1.5-kb *Sal*I-*Bam*HI fragment. These results suggest that the 1.5-kb *Sal*I-*Bam*HI fragment is located at the 5' end of the G6PD mRNA and the 0.45-kb *Ava*I fragment is located near the 3' end of the G6PD mRNA. This leads to the 5'–3' orientation shown in Fig. 2 and is consistent with the results of the S1 nuclease mapping experiment.

(d) Copy number of the G6PD gene in male and female *Drosophila* genomes

As shown above, the steady-state amount of G6PD mRNA is approximately equivalent in both male and female *Drosophila*. One explanation for this would be a single duplication of the G6PD gene in males, thus providing G6PD mRNA equivalence between the sexes.

We therefore examined the average copy number of the G6PD gene sequence in the genomes of male and female *Drosophila*. Genomic DNA from adults was digested with *Pst*I and blotted to nitrocellulose after gel electrophoresis. Included on the Southern blot was DNA from subclone pDmG20/3.9RI, containing exonII equivalent to 1, 2, 4 and 6 copies per haploid female genome (i.e., one set of autosomes and one X-chromosome). When the Southern blot was probed with a 32 P-labeled 1.35-kb *Pst*I fragment containing the 3'-distal half of exonII, the only genomic DNA band that hybridized was of length 1.35 kb (Fig. 8). When the intensities of the hybridization in the genomic DNAs are compared with those of the various equivalents in the cloned DNA, it is apparent that both males and females have one copy of the G6PD gene sequence per X chromosome and that the G6PD gene in the male X-chromosome is not duplicated.

DISCUSSION

(a) Isolation of the G6PD gene sequence

We have been able to select from a *Drosophila* genomic λ library a recombinant phage, λ DmG21, containing the DNA sequence encoding G6PD. Three separate approaches were employed to confirm the presence of G6PD coding sequences in the recombinant λ phage. First, the genomic location of the inserted *Drosophila* DNA fragment was shown by in situ hybridization to correspond to the chromosomal site of G6PD. Second, the in vitro translation product of hybrid-selected mRNA was identical in M_r (i.e., 55000) to the monomeric unit of G6PD. Due to the low abundance of G6PD mRNA in the total RNA population, amounts of hybrid-selected RNA could not be obtained in sufficient quantities

Fig. 8. Determination of the G6PD gene copy number in adult male and female *Drosophila* DNA. Nuclear DNAs (10 μ g each) from adult male (lane a) and female (lane b) flies digested with *Pst*I and electrophoresed on a 1.0% agarose gel. Included in the gel was DNA from subclone pDmG20/3.9RI, 1.0, 2.0, 4.0 and 6.0 female haploid genome equivalents (lanes c–f, respectively). A Southern blot of the gel was hybridized to a radiolabeled 1.35-kb *Pst*I fragment contained in exon II.

to determine whether the 55-kDal translation product is recognized by antisera directed against G6PD. However, digestion of the 3.9-kb *Eco*RI fragment containing exon II with BAL 31 nuclease, followed by insertion of the resected DNA fragments into the expression vector λ gt11 (Young and Davis, 1983), did yield recombinant phages, 20% of which reacted positively with anti G6PD IgG. Collectively, these results indicate that λ DmG21 contains sequences that encode G6PD.

(b) G6PD mRNA

As shown in Fig. 6, the size of the G6PD mRNA in adult male and female flies is 2.0 kb, and the steady-state levels of the mRNAs in the somatic cells of the two sexes are similar. This observation indicates that similar levels of G6PD enzyme activity in the two sexes (Seecof et al., 1969) is due to the steady-state level of G6PD mRNA. Also, it supports the previous conclusions, based on studies using salivary gland chromosomes (Mukherjee and Beermann, 1965), that dosage compensation is the result of increased transcriptional activity of the male X chromosome. Our results do not, however, eliminate differential RNA processing and/or RNA turnover rates as possible mechanisms for generating equivalent levels of G6PD mRNA in the two sexes.

Overexposure of the autoradiogram (Fig. 6) shows the presence of a minor RNA species of length 2.3 kb in both male and female poly(A)⁺ RNA. Since the 2.3-kb RNA hybridizes with exon I and exon II gene probes, it is possible that this RNA represents a minor species of G6PD mRNA. Whether this RNA results from either differential processing of the G6PD primary transcript or a different transcriptional start or stop site from that of the major 2.0-kb mRNA has not been determined. We have observed, however, the 2.3-kb RNA to be present in the poly(A)⁺ RNA from several embryonic stages of development as well as the three larval stages and the pupal stage (R.G. and J.E.M., unpublished observation). In all cases the ratio of the 2.3- and 2.0-kb mRNAs remains unaltered. Thus it is unlikely that this RNA species represents a minor component in one stage of development and a major component in another.

(c) Gene copy number

It is formally possible that the equivalent steady-state levels of G6PD mRNA in the two sexes result from duplication of the G6PD gene sequence in males. To examine this possibility, the copy number of the G6PD gene was determined in total genomic DNA from both male and female flies (Fig. 8). The results of this experiment show that the number of G6PD gene sequences in male and female genomes is directly proportional to the number of X-chromosomes in the two sexes, and that the G6PD gene

is present as a single copy sequence on the X-chromosome. Dosage compensation of G6PD is not, therefore, due to amplification of the G6PD gene in males.

ACKNOWLEDGEMENTS

We thank R. Yamamoto for his help with the immunization of rabbits and D. Peterson for help in cloning in λ gt11 and immunological screening. We also thank Dr. John Lucchesi for helpful suggestions and J. Massey for technical assistance. This investigation was supported by Grant GM 22207 awarded by the National Institute of Health.

REFERENCES

- Anderson, K.P., Stringer, J.R., Holland, J.E. and Wagner, E.K.: Isolation and localization of *Herpes simplex* virus type I mRNA. *J. Virol.* 30 (1979) 805–820.
- Benton, W.D. and Davis, R.W.: Screening λ gt recombinant clones by hybridization to single plaques in situ. *Science* 196 (1977) 180–182.
- Benyajati, C., Wang, N., Reddy, A., Weinberg, E. and Sofer, W.: Alcohol dehydrogenase in *Drosophila*: isolation and characterization of messenger RNA and cDNA clone. *Nucl. Acids Res.* 8 (1980) 5649–5667.
- Brodelius, P., Larsson, P.-O. and Mosbach, K.: The synthesis of three AMP analogues: *N*⁶-(6-aminoheptyl)-adenosine 5'-monophosphate, *N*⁶-(6-aminoheptyl)-adenosine 2', 5'-bisphosphate, and *N*⁶-(6-aminoheptyl)-adenosine 3', 5'-bisphosphate and their application as general ligands in biospecific affinity chromatography. *Eur. J. Biochem.* 47 (1974) 81–89.
- Casey, J. and Davidson, N.: Rates of formation and thermal stabilities of RNA:DNA and DNA:DNA duplexes at high concentrations of formamide. *Nucl. Acids Res.* 4 (1977) 1539–1550.
- DeFlora, A., Morelli, A., Frascio, M., Corte, G., Curti, B., Galliano, M., Gozzer, C., Minchiotti, L., Mareni, C. and Gaetani, G.: Radioimmunoassay and chemical properties of glucose 6-phosphate dehydrogenase and of a specific NADP(H)-binding protein (FX) from human erythrocytes. *Biochim. Biophys. Acta* 500 (1977) 109–123.
- Fouts, D.L., Manning, J.E., Fox, G.M. and Schmid, C.W.: A complex repeated DNA sequence within the *Drosophila* transposable element copia. *Nucl. Acids Res.* 9 (1981) 7053–7064.
- Goldberg, D.A.: Isolation and partial characterization of the *Drosophila* alcohol dehydrogenase gene. *Proc. Natl. Acad. Sci. USA* 77 (1980) 5794–5798.

- Goudswaard, J., Van der Donk, J.A., Noordzij, A., Van Dam, R.H. and Vaerman, J.-P.: Protein A reactivity various mammalian immunoglobulins. *Scand. J. Immunol.* 8 (1978) 21–28.
- Hazellrigg, T., Levis, R. and Rubin, G.M.: Transformation of white locus DNA in *Drosophila*: dosage compensation, zeste interaction, and position effect. *Cell* 36 (1984) 469–481.
- Holmquist, G.: Transcription rates of individual polytene chromosome bands: effects of gene dose and sex in *Drosophila*. *Chromosoma* 36 (1972) 413–452.
- Hori, S.H. and Tanda, S.: Purification and properties of wild-type and mutant glucose 6-phosphate dehydrogenase and of 6-phosphogluconate dehydrogenase from *Drosophila melanogaster*. *Japan. J. Genet.* 55 (1980) 211–222.
- Kazazian Jr., H.H., Young, W.J. and Childs, B.: X-linked 6-phosphogluconate dehydrogenase in *Drosophila*: subunit associations. *Science* 150 (1965) 1601–1602.
- Korge, G.: Dosage compensation and effect for RNA synthesis in chromosome puffs of *Drosophila melanogaster*. *Nature* 225 (1970) 386–388.
- Laemmli, U.K.: Cleavage of structural proteins during assembly of the head of bacteriophage T4. *Nature* 227 (1970) 680–685.
- Lee, C.-Y., Langley, C.H. and Burkhart, J.: Purification and molecular weight determination of glucose 6-phosphate dehydrogenase and malic enzyme from mouse and *Drosophila*. *Analyt. Biochem.* 86 (1978) 697–706.
- Lefevre Jr., G.: A photographic representation and interpretation of the polytene chromosomes of *Drosophila melanogaster* salivary glands, in Ashburner, M. and Wright, T.R.F. (Eds.), *The Genetics and Biology of Drosophila*, Vol. 1a. Academic Press, London, 1976, pp. 31–66.
- Levy, L.S., Ganguly, R., Ganguly, N. and Manning, J.E.: The selection, expression, and organization of a set of head-specific genes in *Drosophila*. *Develop. Biol.* 94 (1982) 451–464.
- Levy, L.S. and Manning, J.E.: Messenger RNA sequence complexity and homology in developmental stages of *Drosophila*. *Develop. Biol.* 85 (1981) 141–149.
- Lucchesi, J.C.: Dosage compensation: transcription-level regulation of X-linked genes in *Drosophila*. *Am. Zool.* 17 (1977) 685–693.
- Lucchesi, J.C., Rawls, J.M. and Maroni, G.: Gene dosage compensation in metafemales (3X:2A) of *Drosophila*. *Nature* 248 (1974) 564–567.
- Lyon, M.F.: Gene action in the X-chromosome of the mouse (*Mus musculus* L.) *Nature* 190 (1961) 372–373.
- Maniatis, T., Fritsch, D.F. and Sambrook, J.: *Molecular Cloning. A Laboratory Manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982.
- Maniatis, T., Hardison, R.C., Lacy, E., Lauer, J., O'Connell, C., Quon, D., Sim, G.K. and Efstratiadis, A.: The isolation of structural genes from libraries of eukaryotic DNA. *Cell* 15 (1978) 687–701.
- Mukherjee, A.S. and Beermann, W.: Synthesis of ribonucleic acid by X-chromosomes of *Drosophila melanogaster* and the problem of dosage compensation. *Nature* 207 (1965) 785–786.
- Muller, H.J., League, B.B. and Offerman, C.A.: Effects of dosage changes of sex-linked genes, and the compensatory effect of other gene differences between male and female. *Anat. Rec.* 51 (Suppl.) (1931) 110.
- Robson, K.J.H., Chandra, T., MacGillivray, R.T.A. and Woo, S.L.C.: Polysome immunoprecipitation of phenylalanine hydroxylase mRNA from rat liver and cloning its cDNA. *Proc. Natl. Acad. Sci. USA* 79 (1982) 4701–4705.
- Rosen, J.M.: Isolation and characterization of purified rat casein messenger ribonucleic acids. *Biochemistry* 15 (1976) 5263–5271.
- Rubin, G.M. and Spradling, A.C.: Genetic transformation of *Drosophila* with transposable element vectors. *Science* 218 (1982) 348–353.
- Scholnick, S.B., Morgan, B.A. and Hirsh, J.: The cloned Dopa decarboxylase gene is developmentally regulated when reintegrated into the *Drosophila* genome. *Cell* 34 (1983) 37–45.
- Seecof, R.L., Kaplan, W.D. and Futch, D.G.: Dosage compensation for enzyme activities in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* 62 (1969) 528–535.
- Shapiro, S.Z. and Young, J.R.: An immunological method for mRNA purification. Application to messenger RNA encoding Trypanosome variable surface antigen. *J. Biol. Chem.* 256 (1981) 1495–1498.
- Southern, E.M.: Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.* 98 (1975) 503–517.
- Spradling, A.C. and Rubin, G.M.: Transposition of cloned P elements into *Drosophila* germ line chromosomes. *Science* 218 (1982) 341–347.
- Spradling, A.C. and Rubin, G.M.: The effect of chromosomal position on the expression of the *Drosophila* xanthine dehydrogenase gene. *Cell* 34 (1983) 47–57.
- Steele, M.W., Young, W.J. and Childs, B.: Genetic regulation of glucose 6-phosphate dehydrogenase activity in *Drosophila melanogaster*. *Biochem. Genet.* 3 (1969) 359–370.
- Stewart, B.R. and Merriam, J.R.: Segmental aneuploidy and enzyme activity as a method for cytogenetic localization in *Drosophila melanogaster*. *Genetics* 76 (1974) 301–309.
- Stewart, B. and Merriam, J.: Dosage compensation, in Ashburner, M. and Wright, T.R.F. (Eds.), *The Genetics and Biology of Drosophila*, Vol. 2d. Academic Press, London, 1980, pp. 107–140.
- Storti, R.V., Coen, D.M. and Rich, A.: Tissue-specific forms of actin in the developing chick. *Cell* 8 (1976) 521–527.
- Thomas, P.S.: Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. *Proc. Natl. Acad. Sci. USA* 77 (1980) 5201–5205.
- Vicira, J. and Messing, J.: The pUC plasmids, an M13mp7-derived system for insertion mutagenesis and sequencing with synthetic universal primers. *Gene* 19 (1982) 259–268.
- Weir, D.M.: *Handbook of Experimental Immunology*, Blackwell, Oxford, 1967.
- Young, R.A. and Davis, R.W.: Efficient isolation of genes by using antibody probes. *Proc. Natl. Acad. Sci. USA* 80 (1983) 1194–1198.
- Young, W.J., Porter, J.E. and Childs, B.: Glucose-6-phosphate dehydrogenase in *Drosophila*: X-linked electrophoretic variants. *Science* 143 (1964) 140–141.