Developmentally regulated alternate modes of expression of the *Gpdh* locus of *Drosophila*

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Immunoblot analyses have been performed on extracts prepared from *Drosophila melanogaster*. Those analyses have revealed two subunit forms of enzyme glycerol 3-phosphate dehydrogenase (GPDH) in larval tissues and in adult abdominal tissues. Thoracic tissue, which accounts for the bulk of the adult GPDH, has only one subunit form, the smaller. The two subunit forms differ by ~2400 daltons. In agreement with previous genetic and biochemical data indicating that this enzyme is encoded by a single structural gene, analyses of extracts prepared from a strain carrying a GPDH null mutation detect no GPDH polypeptides in larvae or adults. Similarly, analyses of extracts prepared from a strain carrying a mutation which produces a GPDH polypeptide that differs in size from wild-type reveal a change in the adult thoracic GPDH polypeptide as well as a change in both GPDH polypeptides found in larvae. Total *Drosophila* RNA prepared from larvae or newly eclosed adults has been translated in a mRNA-dependent cell-free system. GPDH was immunoprecipitated from the translation products and analyzed. Two subunit forms of GPDH were immunoprecipitated from translation products whose synthesis was directed by larval RNA and only one was detected in the polypeptides synthesized from adult RNA. The GPDH polypeptides synthesized *in vitro* are approximately the same size as the corresponding polypeptides found *in vivo*. The relative proportion of total GPDH represented by each subunit form synthesized *in vitro* is similar to those found *in vivo*. These subunit forms of GPDH appear to arise from distinct mRNAs encoded by the single *Gpdh* gene.

Key words: alternate transcripts/developmental regulation/*Drosophila*/glycerol 3-phosphate dehydrogenase

Introduction

The mechanisms which enable developmental regulation of gene expression are not clearly understood, but it is clear that these mechanisms may be complex and may operate at several levels between the transcription of DNA and maintenance of the steady state concentration of gene product. For example, several genes have been characterized as having alternative transcriptional promoters whose selective use results in mRNAs encoded by a single gene but that have different 5' terminal regions (Lai *et al.*, 1982; Benyajati *et al.*, 1983; Carlson and Botstein, 1982; Perlman *et al.*, 1982; Jellinghaus *et al.*, 1982). Additionally, alternative processing events can result in different mature mRNAs being generated from a transcript of a single gene (Rosek and Davidson, 1983; Schwarzbauer *et al.*, 1983). At the other extreme, turnover of the protein product of a gene may be different in

different cell types (Sullivan *et al.*, 1983), as a result of either post-transcriptional or post-translational modifications, or conceivably by changed specificities of the degrading machinery.

Our previous studies of the enzyme glycerol-3-phosphate dehydrogenase (GPDH) have shown that there is a difference between the adult thorax and abdomen in the turnover of GPDH (Sullivan *et al.*, 1983). The majority, ~70%, of the activity or GPDH protein found in an adult fly is in the thorax, largely in the flight muscle. However, of the total GPDH that is being synthesized in an animal, 74% is in the abdomen, primarily in the fat body. This high relative rate of synthesis with relatively low steady state levels must mean that the abdominal enzyme is turning over much more rapidly than GPDH of the thorax. GPDH in larvae appears to resemble the abdominal situation in most respects.

Here we provide evidence that differences between thoracic and abdominal *Gpdh* expression exist at levels in addition to protein turnover. Two sizes of GPDH subunits are found in larvae and adult abdomens, while only one is present in the adult thorax. *In-vitro* translation products of RNA from these sources show a similar difference. Since all available evidence on the biochemical genetics of *Gpdh* in *Drosophila* indicates a single locus (Wright and Shaw, 1969; O'Brien and MacIntyre, 1972a, 1972b, and the data presented here) we interpret these results to indicate that alternative forms of *Gpdh* transcripts are present in the thorax and abdomen of *Drosophila*.

Results

Initial experiments were aimed towards further elucidating changes that occur in GPDH synthesis in larvae and adult *Drosophila melanogaster*. Whole fly extracts were prepared from third instar larvae or newly eclosed adults. These extracts were separated on SDS-polyacrylamide gels and subsequently electroblotted to nitrocellulose membranes and probed immunologically. Specific GPDH anti-serum detects one band on blots of adult fly extract (Figure 1). That band has the same approximate mol. wt., 34 100 daltons, as a GPDH subunit from purified enzyme (Collier *et al.*, 1976). Similar immuno-blots of larval extract, however, reveal two polypeptides (Figure 1). One of these migrates to approximately the same position as adult GPDH, while the other is approximately 2400 kd larger. The amount of immunological probe bound to each polypeptide suggests that each type of GPDH subunit is represented nearly equally in larval extracts.

To determine whether the larger polypeptide detected is indeed GPDH, or simply the result of cross-reactivity with our anti-sera, we performed the following experiments. Several strains of *Drosophila* were obtained that carry mutations at the *Gpdh* locus. These mutations are described by Kotarski *et al.* (1983a, 1983b). One of these mutants, JH231, was described as lacking both GPDH activity (GPDH⁻) and GPDH cross-reacting material (CRM⁻) (Bewley and Lucchesi, 1977). The absence of GPDH activity has been confirmed in our laboratory (data not shown). Whole-fly extracts were subjected to immuno-blotting and compared with wild-type extracts (Figure 2). Adult extracts contained

Fig. 1. Immuno-blot analysis of wild-type adult and larval *Drosophila* homogenates. Ten individuals were homogenized in 10 mM NaPO₄ (pH 7.5), 0.1 mM EDTA, 2% phenyl thiourea. 5 μ l of the homogenate was electrophoresed through a 12.5% SDS-polyacrylamide gel and transferred to a nitrocellulose membrane. GPDH-specific antisera detects one polypeptide in adult homogenates (A) and two polypeptides in larval homogenates (L). Protein standards were obtained from BRL and are 68 000 BSA, 43 000 ovalbumin, 25 700 α chymotrypsinogen, 18 400 β lactoglobulin, and 12 300 cytochrome C.

no detectable GPDH. Larval extracts had neither GPDH polypeptide detectable in wild-type flies. Increased amounts of fly extract were subjected to this analysis up to levels representing a 4-fold increase over wild-type preparations. In all instances no GPDH can be detected.

Similar analyses have been applied to extracts prepared from another strain, GL3, which also has a mutation in the *Gpdh* locus. This mutation results in the production of a polypeptide that retains enzymatic activity, but differs slightly from that of wild-type in that a large GPDH subunit is produced (Kotarski *et al.*, 1983a). Extracts prepared from larvae prepared for the allele, GL3, contain two polypeptides that are detectable using anti-GPDH sera, as do wild-type preparations. However, each of these polypeptides is slightly larger than the corresponding GPDH subunit found in wild-type (Figure 2). These results, taken together with the data from the strains carrying null mutations, support the hypothesis that both polypeptides arise from the same gene and are thus two forms of GPDH.

To determine whether the two forms of GPDH are differentially present in different tissues, dissection experiments were performed. Extracts were made from adult heads, thoraxes and abdomens. Immuno-blotting analyses of these preparations reveal the characteristic single adult polypeptide present in head and thorax. Abdominal preparations, however, contain the two GPDH

Fig. 2. Immuno-blot analyses of adult and larval homogenates prepared from wild-type and mutant strains of *Drosophila* (GL3 and JH231). The left-hand plate shows an analysis of 5 μ l samples of wild-type and GL3 extracts. The right-hand plate shows an analysis of wild-type and JH231 extracts. Each pair of lanes in the right hand plate contains 5 μ l and 10 μ l, respectively, of the indicated homogenates. Lanes labeled L contain larval homogenates and lanes labeled A contain adult homogenates.

forms. That seen as the adult form is the predominant one, but smaller amounts of the form characteristic of larvae are also detectable (Figure 3). Since abdominal GPDH represents only ~ 15% of whole fly GPDH (Sullivan *et al.*, 1983), the larger form is not usually detected on immuno-blot of whole flies (Figure 1). Experiments using homogenates prepared from dissected body segments of male or female flies reveal no detectable differences between them.

Similar immuno-blot analyses were carried out on specific tissues dissected out of adult abdomens and larvae. Adult gut, Malpighian tubules, reproductive tissue (male or female), and abdominal cuticle were analyzed for GPDH. All of these tissues contained detectable amounts of both GPDH subunit forms. Larval gut, Malpighian tubules, fat body, salivary glands and integument were also analyzed. GPDH was detected in gut, fat body and Malpighian tubules. Again, each of these tissues contained both GPDH subunit forms.

The experiments described above reveal a difference in GPDH subunit forms that is manifested at the level of the protein in a developmental stage and tissue specific manner. To determine whether these different forms arise from two different messenger RNAs or perhaps from differential translation of a single mRNA, *in-vitro* translation experiments were performed. Total RNA was isolated from third instar larvae and newly eclosed adults. The RNAs were then translated *in vitro* in a message-dependent rabbit reticulocyte cell-free translation system. The labeled polypeptides were separated on SDS-polyacrylamide gels and visualized

Fig. 3. Immuno-blot analysis of homogenates prepared from dissected body segments. Heads (H), thoraxes (T) and abdomens (A) were dissected from 10 individuals and homogenized in 50 μ l of grinding buffer. 10 μ l of each homogenate was subjected to immuno-blot analysis.

by fluorography (Figure 4). Immunoprecipitation of GPDH synthesized *in vitro* reveals only one GPDH polypeptide when synthesis is directed by adult RNA. This polypeptide is 34 683 daltons $\pm$ 300 s.d., $n = 12$. The GPDH detected by immuno-blot analysis of adult fly extracts is within this range. The analysis of GPDH polypeptides, when synthesis is directed by larval RNA, reveals two immunoprecipitable polypeptides. One has a mol. wt. of 37 162 $\pm$ 380 s.d., $n = 12$. The other has a mol. wt. of 34 290 $\pm$ 160 s.d., $n = 12$. The size of the polypeptides detected by immuno-blot analyses are not significantly different from the two GPDH forms synthesized from larval RNA and appear to be present in relative amounts similar to those found *in vivo* (Figure 4). These results indicate that the two GPDH subunit forms found in larval-fly extracts are probably encoded by two mRNAs. Additionally, the similarity observed between those polypeptides found *in vivo* and those synthesized *in vitro* indicates that there is no detectable post-translational processing occurring with these GPDH polypeptides.

RNA was also prepared from larvae of the mutant strains JH231 and GL3. Translation of these RNAs *in vitro* and subsequent immunoprecipitation of the translation products give the results presented in Figure 5. RNA isolated from JH231 failed to direct the synthesis of any GPDH polypeptides. The RNA isolated from larvae of GL3 directed the synthesis of two immunoprecipitable polypeptides, each slightly larger than the corresponding wild-type polypeptide. The polypeptides synthesiz-

Fig. 4. Fluorograph of GPDH immunoprecipitated from polypeptides synthesized *in vitro*. Total RNA purified from mid-third instar larvae and newly-eclosed adults was translated in an mRNA-dependent cell-free translation system. GPDH was immunoprecipitated from the translation products and displayed on an SDS gel. The smallest polypeptide in the larval lane is the result of contamination by a major larval translation product with a mol. wt. of $\sim$ 20 000. Protein standards 14 C-labeled were obtained from BRL. They are phosphorylase B, 92 500; BSA, 68 000; ovalbumin, 43 000; chymotrypsinogen, 24 700; β lactoglobulin, 18 700 and cytochrome C, 12 300.

ed by GL3 larval RNA appeared to be identical to the ones found *in vivo* and detected by immuno-blotting of extracts of larvae homozygous for this mutant allele.

Discussion

We interpret the results presented here to indicate that two mRNAs encoding the enzyme GPDH are present in larvae and to a lesser extent in adult abdomens, while only one is present in adult thorax. The evidence for this conclusion is based primarily on immunological data, obtained by probing polypeptides synthesized either *in vitro* or *in vivo* with GPDH-specific antisera. Several control experiments were performed to exclude the possibility that the two GPDH polypeptides we detect are the result of immunological artifact. Anti-sera independently prepared in several different rabbits or in a goat from several independent preparations of antigen were used to immunoprecipitate *in-vitro* translation products. In all cases the results were exactly as presented here. Reactions with non-immune antisera failed to immunoprecipitate any detectable *Drosophila* polypeptide from either *in-vitro* translation products or proteins synthesized *in vivo*.

We have considered the possibility that the two GPDH polypeptides are synthesized from one mRNA and differ by a post-translational processing event or that one serves as a precursor of the other. These possibilities seem highly unlikely. All components in our *in-vitro* translation system remain the same except for the *Drosophila* RNA that is added. This system produces one polypeptide that precipitates with our GPDH-specific antisera when

Fig. 5. Fluorograph of total translation products and immunoprecipitated GPDH. Total RNA was isolated from wild-type adults synchronous at eclosion, mid-third instar larvae, and larvae of strains homozygous for the *Gpdh* mutations GL3 and JH231. The RNA was translated *in vitro* and the translation products displayed on an SDS-polyacrylamide gel. The **left-hand plate** shows the total translation products synthesized from endogenous mRNAs. The **right-hand plate** shows the GPDH polypeptides immunoprecipitated from *in-vitro* translations directed by the same RNAs. The immunoprecipitates were not washed extensively so that the background bands could be used to align gel lanes for comparison. The position of the wild-type adult GPDH subunit form is indicated by the arrow. Protein standards as in Figure 4.

supplemented with RNA prepared from newly eclosed adults and two polypeptides when supplemented with larval RNA. The differences observed in polypeptides synthesized *in vitro* are therefore due to differences intrinsic to the RNA added to the system. With this in mind it is unlikely that either GPDH polypeptide synthesized by larval RNA arises as a result of post-translational modification of the other polypeptide. Additionally, post-translational modification in an unsupplemented rabbit reticulocyte cell-free translation system is not known to occur. Furthermore, the modification would have to occur both in our *in-vitro* translation system and *in vivo* in a stage specific fashion.

The existence of only one gene encoding GPDH in *Drosophila* is of course essential for our interpretation of the data presented here. This evidence has been reviewed by Bewley (1983). In summary it is as follows. Naturally occurring electrophoretic variants of GPDH have been described by O'Brian and MacIntyre (1972a, 1972b). All isozymic forms of GPDH have altered electrophoretic

mobility. Wright and Shaw (1969) analyzed the GPDH produced in animals heterozygous for these alleles and observed the expected heterodimers having mobilities intermediate between the two parental allozymes. Null mutants have also been isolated that lack all GPDH activity yet still produce GPDH cross-reacting material (Bewley and Miller, 1979). These point mutants affect all GPDH isozymes, indicating that the observed GPDH isozymes are encoded by a single gene. In later studies, Kotarski *et al.* (1983a, 1983b) analyzed the chromosomal region around the *Gpdh* locus as well as many *Gpdh* mutants. Those studies mapped the *Gpdh* locus cytogenetically to position 25F5, a single polytene chromosome band. Taken together, then, there is overwhelming evidence indicating that there exists only one gene encoding GPDH in *Drosophila*.

Clearly the most convincing demonstration that both polypeptides are products of the *Gpdh* locus is the alteration of polypeptide size in animals homozygous for the *Gpdh* allele, GL3. In

these animals each GPDH polypeptide has an increased size. It should be noted that in the original description of GL3 (Kotarski *et al.*, 1983b) the GPDH subunits were thought to have been shorter than the wild-type GPDH subunit. To determine whether the apparent contradiction of our results with the previous study could have been due to difference between wild-type lines, we examined GPDH polypeptides of several wild-type strains including the line from which GL3 was derived. The size of the GPDH subunits in all wild-type lines is identical. We have been in contact with the laboratory in which GL3 was isolated and characterized. There is agreement between us that the mutation GL3 does result in the production of GPDH polypeptide that is ~2–3% larger than wild-type GPDH (R.J. MacIntyre, personal communication). In any case this difference does not alter our interpretation that the mutation, GL3, alters the size of both GPDH polypeptides.

It has been known for some time that isozymic forms of native GPDH are found in *Drosophila* (Wright and Shaw, 1969; Bewley and Luccesi, 1977). While we have no direct evidence to connect our observations of two GPDH subunit forms which differ by mol. wt. from the previously described electrophoretic isozymes, several interesting correlations between these phenomena are evident. The isozyme, GPDH-3, is found primarily in larvae (Wright and Shaw, 1969; Bewley and Luccesi, 1977), when we find the larger of the two polypeptides is most abundant. GPDH-3 is absent from young adult flies and in particular is not found in dissected thoracic flight muscle (Wright and Shaw, 1969) where we find only one GPDH polypeptide, the smaller one. GPDH-3 is also found in older adult flies in low amounts. We also find evidence of the larger GPDH polypeptide in low amounts in the abdomens of mature adults. Results of Niesel *et al.* (1980, 1982) have indicated that GPDH-3 contains subunits that are shorter by three amino acids as compared with the subunits of GPDH-1. Our results offer no contradiction to those data. In fact it consistently appears in both immuno-blots and in the translation products that the smaller larval GPDH subunit is slightly smaller than the adult thoracic GPDH subunit. This difference is not statistically significant, however.

The mechanism responsible for the generation of the two GPDH polypeptides remains to be elucidated. They could result from either alternative transcription or mRNA processing events. It is even conceivable that the longer GPDH polypeptide found in larvae and adult abdomens does not associate to form dimers that are enzymatically active. However, we are confident that this peptide is a product of the *Gpdh* locus. Analysis of cloned *Gpdh* DNA should in the future provide a basis for understanding the mechanism through which this locus generates alternative transcripts in a developmentally specific fashion.

Materials and methods

SDS-polyacrylamide gels

SDS-Polyacrylamide gel electrophoresis was performed according to the procedures described by Laemmli (1970). Following electrophoresis, gels containing radioactively labeled polypeptides were prepared for fluorography by treatment with Enhance (New England Nuclear) according to the instructions supplied by the manufacturer. Following treatment, gels were dried and exposed to pre-exposed X-ray film (Laskey and Mills, 1975).

Immuno-blot analyses

Immuno-blot analyses were carried out essentially according to the procedures of Towbin *et al.* (1979) and Burnette (1981) with some modifications. GPDH-Specific rabbit IgG, described previously (Sullivan *et al.*, 1983), bound to GPDH immobilized on nitrocellulose membrane was visualized with goat anti-rabbit IgG conjugated to horseradish peroxidase (BioRad) according to the instructions provided by the supplier.

RNA preparation

Total RNA was prepared from either 4-day-old larvae, adults synchronous at emergence, or adults synchronous at five days post-eclosion as indicated. Flies were ground in liquid nitrogen to a fine powder. That powder was suspended in 15 volumes of 8 M guanidine-HCl and homogenized with two passes in a glass-Teflon homogenizer. The homogenate was cleared by centrifugation for 15 min at 20 000 g. The resultant supernatant was filtered through nitex cloth (25 µm pore), and precipitated by adding a half volume of 95% ethanol and cooling to –20°C. The precipitate was suspended in 7.5 vols of 4 M guanidine-HCl and reprecipitated with a half volume of 95% ethanol. This was repeated once with 3.75 vols to 4 M guanidine-HCl. The final precipitate was extracted with 10 vols of 150 mM NaCl at 56°C, three times, each time the volume of NaCl was decreased by one half. The supernatants from these extractions were pooled and the RNA was precipitated by the addition of 2 vols of 95% ethanol and cooling to –20°C. When needed, the precipitated RNA was removed by centrifugation and dissolved in a convenient volume of sterile distilled water.

Rabbit reticulocyte cell-free translation system

The mRNA-dependent rabbit reticulocyte lysate cell-free translation system was prepared according to procedures described by Pelham and Jackson (1976) and Paterson and Roberts (1981) with some modifications. Translation of *Drosophila* RNA was carried out in a reaction volume of 13 µl which consisted of 5 µl nuclease treated reticulocyte lysate, 4 µl of supplement mixture (65 mM Hepes, pH 7.6, 6.5 mM dithiothreitol, 26 mM creatin phosphate, 1.63 mM spermidine (free base) and 227.5 µM of each of the 19 essential amino acids excluding methionine), 1 µl of a solution containing 1.83 mM potassium acetate and 2.6 mM magnesium acetate, 1 µl of 35S-methionine, and 2 µl of RAN (1–2 µg). The translation mixture was incubated at 30°C for 75 min. 1 µl of the translation reaction was routinely prepared for electrophoretic analysis of the polypeptides synthesized.

Immunoprecipitation of GPDH synthesized in vitro

Immunoprecipitation of GPDH was carried out as previously described (Sullivan *et al.*, 1983). 10 µl of the *in-vitro* translation reaction was typically used in the reaction. The immunoprecipitate was subjected to electrophoresis on SDS-polyacrylamide gels and visualized by fluorography.

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References

- Benyajati, C., Spoerel, N., Haymerle, H. and Ashburner, M. (1983) *Cell*, **33**, 125–133.
- Bewley, G.C. (1983) in Rattazzi, M.C., Scandalios, J.G. and Whitt, G.S. (eds.), *Isozymes: Current Topics in Biological and Medical Research*, Alan R. Liss, N.Y., Vol. 9, pp. 33–62.
- Bewley, G.C. and Lucchesi, J.C. (1977) *Biochem. Genetics*, **15**, 235–251.
- Bewley, G.C. and Miller, S. (1979) in Rattazzi, M.C., Scandalios, J.G. and Whitt, G.S. (eds.), *Isozymes: Current Topics in Biological and Medical Research*, Alan R. Liss, N.Y., Vol. 3, pp. 23–52.
- Burnette, W.N. (1981) *Anal. Biochem.*, **112**, 195–203.
- Carlson, M. and Botstein, D. (1982) *Cell*, **28**, 145–154.
- Collier, G.E., Sullivan, D.T. and MacIntyre, R.J. (1976) *Biochim. Biophys. Acta*, **429**, 316–323.
- Jellinghaus, U., Schatzle, U., Schmid, W. and Roewekamp, W. (1982) *J. Mol. Biol.*, **159**, 623–636.
- Kotarski, M.A., Pickert, S. and MacIntyre, R.J. (1983a) *Genetics*, **105**, 371–386.
- Kotarski, M.A., Pickert, S., Leonard, D.A., LaRosa, G.J. and MacIntyre, R.J. (1983b) *Genetics*, **105**, 387–407.
- Laemmli, U.K. (1970) *Nature*, **227**, 680–685.
- Lai, E.C., Roop, D.R., Tsai, M.J., Woo, S.L.C. and O'Mally, B.W. (1982) *Nucleic Acids Res.*, **10**, 5553–5567.
- Laskey, R.A. and Mills, A.D. (1975) *Eur. J. Biochem.*, **56**, 335–341.
- Niesel, D.W., Bewley, G.C., Miller, S.G. and Armstrong, F.B. (1980) *J. Biol. Chem.*, **255**, 4037–4080.
- Niesel, D.W., Pan, Y.-C.E., Bewley, G.C., Armstrong, F.B. and Li, S.S.-L. (1982) *J. Biol. Chem.*, **257**, 979–983.
- O'Brien, S.J. and MacIntyre, R.J. (1972a) *Biochem. Genet.*, **7**, 141–161.
- O'Brien, S.J. and MacIntyre, R.J. (1972b) *Genetics*, **71**, 127–138.
- Paterson, B.M. and Roberts, B.E. (1981) in Chirikjian, J.G. and Papas, T.S. (eds.), *Gene Amplification and Analysis*, Elsevier/North-Holland, NY, Vol. 2, 417–437.
- Pelham, H.R.B. and Jackson, R.J. (1976) *Eur. J. Biochem.*, **67**, 247–256.
- Perlman, D., Halvorson, H.O. and Cannon, L.E. (1982) *Proc. Natl. Acad. Sci. USA*, **79**, 781–785.

- Rosek, C.E. and Davidson, N. (1983) *Cell*, **32**, 23-34.
- Schwarzbauer, J.E., Tamkun, J.W., Lemischka, I.R. and Hynes, R.O. (1983) *Cell*, **35**, 421-431.
- Sullivan, D.T., Donovan, F.A. and Skuse, G. (1983) *Biochem. Genet.*, **21**, 49-62.
- Towbin, H., Staehelin, T. and Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA*, **76**, 4350-4354.
- Wright, D.A. and Shaw, C.R. (1969) *Biochem. Genet.*, **3**, 343-353.

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