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Regulation and Expression of α-Glycerol-3-phosphate Dehydrogenase (GPDH) in *Drosophila melanogaster*

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Several parameters of α-glycerol-3-pholphate dehydrogenase (GPDH) such as activity, content and translatable mRNA levels were measured to elucidate mechanism underlying developmental and tissue specific regulation of GPDH activity in *Drosophila melanogaster.* In adult segments, most of total GPDH activity (62%) was detected in thorax where GPDH-1 resided, while 32% of total GPDH activity was only detected in abdomen where GPDH-3 resided. The relative synthesis of GPDH was, however, similar in both tissues, although 58% of total GPDH was synthesized in abdomen. These results strongly suggest that the turnover rate of the abdominal enzyme (GPDH-3) was much more rapid than that of thoracic enzymes (GPDH-1). *In vitro* translation and immunoblotting experiments also indicate that GPDH-3 was arised by posttranslational modification from a single polypeptide (GPDH-1).

KEY WORDS: **α-Glycerol-3-phosphate dehydrogenase, Developmental regulation, *Drosophila***

The differential expression of a specific gene in both space and time is of fundamental importance in elucidating the α-glycerol-3-phosphate dehydrogenase (GPDH, NAD^+ oxidoreductase, E. C. No. 1.1.1.8) in *Drosophila melanogaster* represents one of the ideal system in examining gene expression during development (O'Brien and MacIntyre, 1972a; Bewley and Miller, 1979; Kim *et al.*, 1987). Its activity is associated with three isozymic species such as GPDH-1, GPDH-2 and GPDH-3 that are uniquely distributed with respect to developmental and tissue specificity (Grell, 1967; Wright and Shaw, 1969).

Intensive genetic and biochemical analyses suggest that the primary structure of each isozyme is nearly identical: a homodimer with subunit Mr. 31,700 (Bewley and Miller, 1979; Miller and Berger, 1979; Niesel *et al.*, 1980; Niesel *et al.*, 1982). The structural gene, $Gpdh^+$, is localized in the cytogenetic map region 25F-26B on the left arm of the second chromosome (Grell, 1967; O'Brien and MacIntyre, 1972b; Wilkins *et al.*, 1982; Kotarski *et al.*, 1983). However, little is known about the entire spectrum of developmental regulation of GPDH expression. This study was, therefore, performed to elucidate the developmental regulation and the origin of GPDH isozymes in *Drosophila melanogaster.*

Materials and Methods

Culture of *Drosophila melanogaster*

Oregon-R wild type stock of *Drosophila melanogaster* was maintained in uncrowded half-pint bottles on standard cornmeal-sucrose-yeast-agar medium containing propionic acid as mold inhibitor. Culture medium was supplemented with live

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yeast and maintained at 25°C. The flies were grown in mass culture at 25°C and 65% humidity when needed (Mitchell and Mitchell, 1964). The developmental stages of *Drosophila* were identified according to Ashburner and Tompson (1978).

Enzyme activity assay

The α-glycerol-3-phosphate dehydrogenase (GPDH) activity was measured by monitoring the reduction of NAD^+ at 340 nm according to the procedure of McDonald and Avise (1976) with a minor modification. Enzyme activities were expressed as $\Delta OD/min \times 10^2 \pm$ S. D. Protein contents were determined by the method of Lowry *et al.* (1951).

Purification of GPDH and immunological procedure

The GPDH-1 was purified by heat denaturation (25min at 50°C), ammonium sulfate fractionation (40-60%) and followed by flat-bed electrofocusing in granulated gel (Winter *et al.*, 1980).

The GPDH antiserum was prepared in New Zealand white rabbit (Bailey, 1984). Quchterlony double diffusion was conducted in 1% agarose gel containing 50 mM Tris-HCl, pH 8.0. The rocket immunoelectrophoresis was performed by a modified method of Laurell (1966). After electrophoresis, the gel plate was extensively washed in cold 100mM Tris-HCl (pH 8.6), and stained by adding 5 mg NAD^+, 9.7mg glycerol-3-phosphate, 5mg MTT and 1mg PMS. The area under each rocket was approximated by multiplying the rocket height in millimeters by the rocket width at half-height. A standard calibration was linear with a serial dilution of crude antigen.

Radiolabeling of Proteins with [^{35}S]methionine

For labeling of adult flies, 15 flies were placed in a small, dry vial for 30 min. They were then transferred to second vial that contained a filter paper disk saturated with 1% sucrose solution containing 100 μCi/ml [^{35}S]methionine (Amersham). The vial was kept in the dark at 25°C for 70min, the labeled tissues were prepared for electrophoretic analysis.

Electrophoretic procedures

SDS-polyacrylamide gel electrophoresis was conducted according to Laemmli (1970). Two-dimensional gel electrophoresis was performed by the procedure of O'Farrell (1975) with a minor modification. For the fluorography, the gels were impregnated with diphenyl oxazole (PPO), dried and exposed to Kodak XR film, as described by Laskey and Mills (1975).

Immunoblot analysis

Immunoblot analysis was carried out essentially according to the procedures of Towbin *et al.* (1979). The GPDH-specific rabbit IgG bound to GPDH immobilized on nitrocellulose membrane was visualized with goat anti-rabbit IgG conjugated to horseradish peroxidase (HRP) according to the instruction provided by the supplier.

RNA extraction and *in vitro* translation

Total RNA was prepared according to the method described by Jowett (1986). The final precipitated RNA was removed by centrifugation and dissolved in a convenient volume of sterile distilled water. RNA concentration was determined by absorption at 260 nm. Translation was carried out in a reaction volume of 13 μl of a rabbit reticulocyte translation system (Amersham) containing 1 μl of [^{35}S]methionine and 2 μl of RNA according to manufacturer's instructions. The translation mixture were incubated at 30°C for 90 min.

Results

Immunological analysis of GPDH isozymes

The α-glycerol-3-phosphate dehydrogenase (GPDH) isozymes showed the tissue and developmental specific expression patterns (Fig. 1). GPDH-1, the predominant isozymic species in adult, was associated with the thorax segment, while GPDH-3 isozyme was localized in larval tissues and abdomen segment of adult. For further analysis of GPDH isozymes, GPDH-1 was purified (Fig. 2). The immunological identification of GPDH isozymes with anti-GPDH-1 antiserum was determined by a Quchterlony double diffusion. Fig. 3 showed that GPDH-1 and GPDH-3 were coprecipitated, indicating that two isozymes may be antigenically similar.

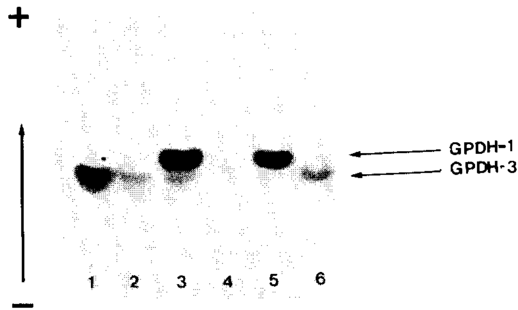

Fig. 1. Developmental and tissue specific expression of α -glycerol-3-phosphate dehydrogenase (GPDH) isozymes in *Drosophila melanogaster.* The experssion of each isozyme was illustrated by agarose gel electrophoresis on citric acid-histidine-NaOH buffer (pH 7.0). Lane 1, Whole organism of third instar larvae; 2, prepupa; 3,5 days adult; 4, head segments; 5, thorax segments; 6, abdomen segments of 5 day adult.

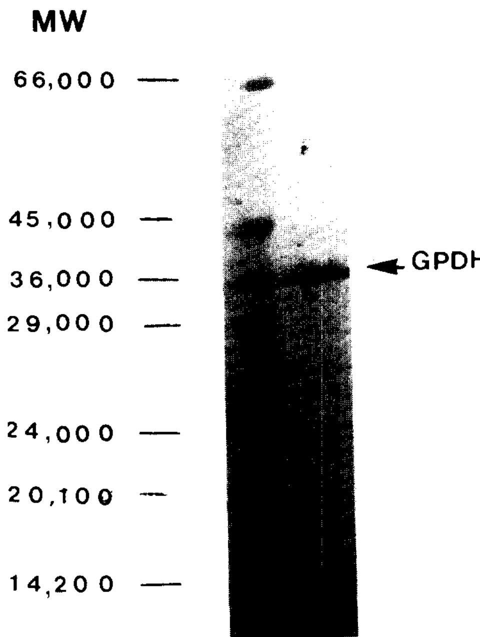

Fig. 2. SDS-polyacrylamide gel (12.5%) showing the subunit molecular weight of purified α -glycerol-3- -phosphate dehydrogenase. The molecular weight standards were bovine seurm albumin (66,000), ovalbumin (45,000), rabbit glyceraldehyde-3-phosphate dehydrogenase (36,000), carbonic anhydrase (29,000), trypsinogen (24,000), soybean trypsin inhibitor (21,000) and α -lactalbumin (14,200).

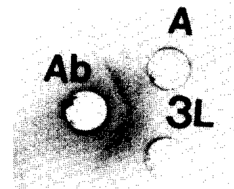

Fig. 3. Immunological analysis of α -glycerol-3-phosphate dehydrogenase (GPDH) isozymes. Quchterlony double diffusion plate of Coomassie brilliant blue-R250 stained precipitin lines formed by 24 hrs of incubation at room temperature. Well (Ab) contains 5 μl of rabbit anti-GPDH-1 antiserum. Well (A) contains adult extracts preincubated at 50°C for 25 min (GPDH-1). Well (3L) contains third instar larval extracts (GPDH-3).

Developmental and tissue specific distribution of GPDH activity

The developmental changes in GPDH activity were compared with those in GPDH content determined by rocket immunoeletrophoresis (Fig. 4 and 5). In the third instar larvae, 5-6 days hatching, the GPDH activity reached a peak and then declined sharply during the early stage of pupal development. From the late pupal stage, this activity increased steadily, and reached at 3-5 times higher level than those observed in late third instar larvae and early pupae. Since the level of translatable GPDH mRNA during development was consistent with that of GPDH activity (Fig. 5; see insert), the developmental profile of GPDH activity appears to be mainly due to transcriptional activity of GPDH gene. There was a slight difference in GPDH activity and GPDH content. From

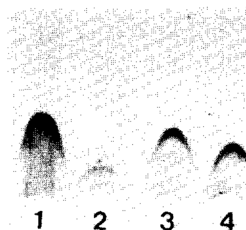

Fig. 4. Typical rocket immunoelectrophoresis. Ten individuals were homogenized in 10 mM sodium phosphate (pH 7,5), containing 1 mM DTT, 1 mM EDTA, and 0.1 mM PMSF. Five μl of homogenates were applied on each well. Well 1, 5 days adult; well 2, head; well 3, thorax;, well 4, abdomen segments of 5 days adult.

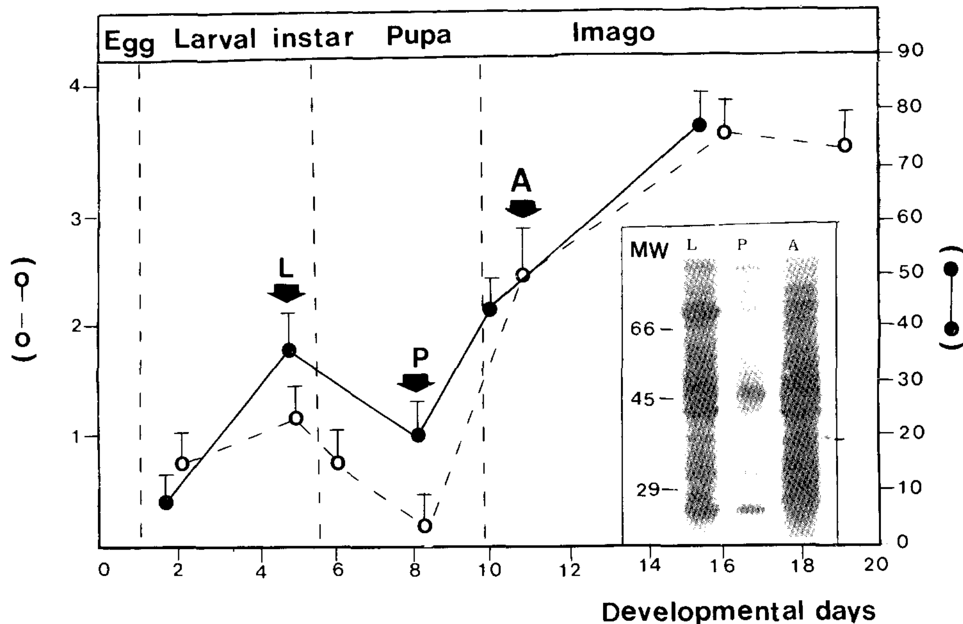

Fig. 5. The developmental changes of GPDH activities and GPDH contents in *Drosophila melanogaster*. GPDH activities and GPDH contents were measured by using 10 μl of 23,000 $\times$ g supernatant of homogenates from ten individuals. (○——○) GPDH activities per organism (ΔOD/min $\times$ 10^2), abscissa left; (●——●) GPDH contents per organism (rocket height $\times$ rocket width at half-height in millimeter), abscissa right. Results were the average of four replicates $\pm$ S.D. Insert; The developmental changes of translatable GPDH mRNA level. Total RNA were extracted from the each developmental stages (indicated by arrows), translated *in vitro* as described in Materials and Methods. The translation products were displayed on SDS-polyacrylamide gel, and detected by fluorography. The position of GPDH subunit was identified by comparing with that of purified GPDH on the same gel.

the late third instar larvae to 5 days imago with steady state level, GPDH content was doubled, while GPDH activity was increased 3 times. These results indicate the possiblity that the turnover of GPDH in larval GPDH may be higher than that in adult GPDH, or two isozymes may be different in catalytic properties.

Regulation of GPDH activity in different tissues

Because both of two isozymes were experssed in adults, cell type-specific control of GPDH activity was then investigated in thorax and abdomen segments. In adult segments, most of total GPDH activity (62%) was detected in thorax, where GPDH-1 was expressed, while head and abdomen segments containing GPDH-3 were 6.4% and 32% of the total GPDH activity (Fig. 6 and Table 1). Although the GPDH content measured in thorax was merely 58% of total GPDH content, the contribution in GPDH activity by this segment was nearly 62% of the total activity (Table 1). However, the relative synthesis of GPDH was similar in both tissues, although 58% of total GPDH was synthesized in abdomen. This high relative rate of synthesis with a relatively low steady state level indicates that turnover of the abdominal enzymes was more rapid than that of thoracic counterpart.

Origin of GPDH isozymes

To investigate whether each GPDH isozymic species is arised from transcriptional level or post-translational modification, *in vitro* translation experiments were performed. Total RNAs from the third instar larvae and adult segments were translated *in vitro* and the translation products were separated on SDS-polyacrylamide gel and visualized by immunoblotting (Fig. 7). Only one GPDH polypeptide with Mr. of about 32,000 was detected in larval and adult RNAs. These translation

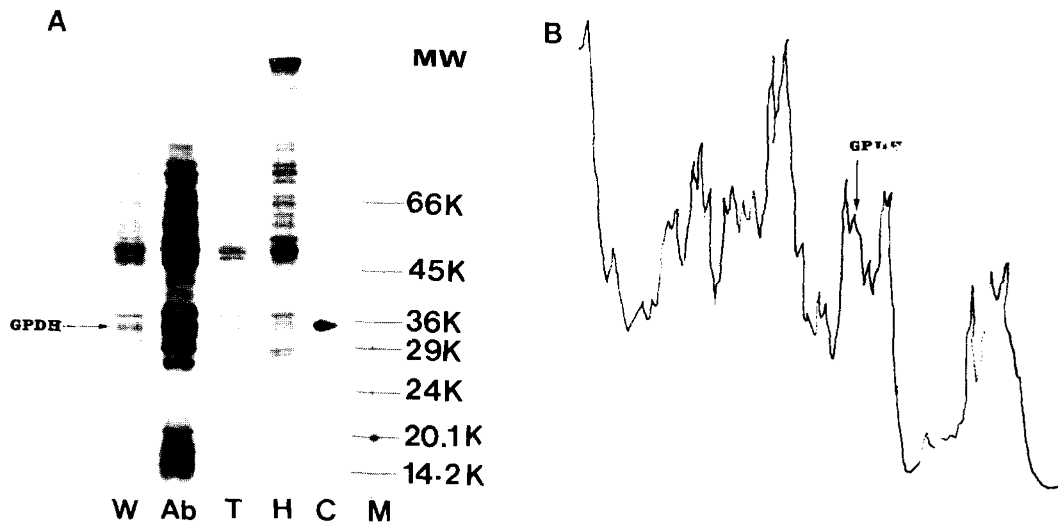

Fig. 6. The relative rates of α-glycerol-3-phosphate dehydrogenase (GPDH) synthesis in adult segments. Ten intact flies were fed [^{35}S]methionine and the parts dissected from these flies were electrophoresed on SDS-polyacrylamide gel (8-15% gradient). (A) Proteins were detected by fluorography. M, molecular weight standards; C, purified GPDH; H, head; T, thorax; Ab, abdomen; W, whole adult. Arrow indicates the position of GPDH subunit. (B) Typical gel scanning of fluorograms in (A).

Table 1. GPDH activities, contents and synthesis in adult segments of *Drosophila melanogaster*.

Segments	GPDH[a] activities	GPDH[b] contents	Relative[c] synthesis of GPDH	Percent of[c] total GPDH synthesis
Head	0.23 ± 0.06(6.4%)	12.6 ± 0.9(16.4%)	1.8%	9.3%
Thorax	2.20 ± 0.14(61.1%)	40.4 ± 1.0(53.0%)	10.8%	32.8%
Abdomen	1.15 ± 0.12(32%)	23.6 ± 1.1(30.6%)	8.0%	57.9%

[a]GPDH activities were expressed $\triangle$OD/min × 10^2(± S. D.) per animal part.

[b]GPDH contents were expressed as rocket height × rocket width at half-height in millimeter.

[c]Intact 5 days-old adult were fed [^{35}S]methionine and the parts dissected from these files were electrophoresed. The relative rates of GPDH synthesis were measured by densitometry of resulting fluorograms (Fig. 6). Results were the average of four replicates ± S. D.

products also showed only one isozymic form on starch gel electrophoresis (Fig. 8). These results indicate that the GPDH-3 may be arised by a specific posttranslational modification of GPDH-1.

Discussion

The distribution of major isozymes of α-glycerol-3-phosphate dehydrogenase (GPDH) in *Drosophila melanogaster* was unique with respect to developmental stage, tissue specific localization,

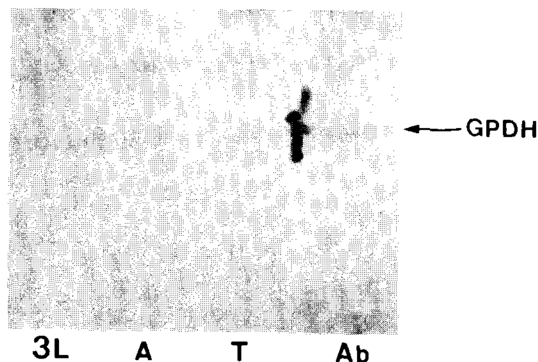

Fig. 7. Immunoblot analysis of GPDH subunit from translation products synthesized *in vitro*. Total RNAs purified from third instar larvae and adult segments were translated in a mRNA-dependent cell free translation system. Translation products were electrophoresed on SDS-polyacrylamide gel (8-15% gradient) and transferred to a nitrocellulose membrane. GPDH subunit was detected as described in Materials and Methods. 3L, third instar larvae; A, whole adults; T, thorax segments; Ab, abdomen segments dissected from 5 days adults.

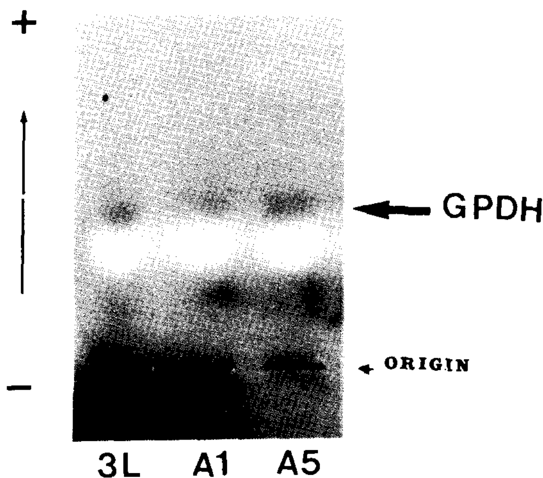

Fig. 8. GPDH activity stain of *in vitro* translation products synthesized by total RNA after starch gel electrophoresis on citric acid-histidine-NaOH (pH 7.0). (3L), 4 μg of third instar larval RNA; (A1), 6 μg of 1 day adult RNA; (A5), 6 μg of 5 days adult RNA. Only one isozymic form (GPDH-1) was detected in all lanes.

physicochemical and kinetic parameter (Bewley and miller, 1979; Niesel *et al.*, 1980, 1982; Skuse and Sullivan, 1985). GPDH-1 and GPDH-3 were antigenically very similar each other. This result indicates that there is a little difference in structure of two isozymes.

A comparison among the activity, content, and relative synthesis of GPDH in adult segments suggests the existence of differential regulation mechanism of GPDH activity. The data obtained in this study also showed the cell type-specific control of GPDH activity, in particular, thoracic musculature and fat body containing the majority of an animal's GPDH. The turnover of the product of *Gpdh*$^+$ locus in the fat body (GPDH-3) was characterized as being more rapid than that of thoracic muscle. Sullivan *et al.* (1983) previously reported that there was a difference in the turnover of GPDH between thorax and abdomen of the adult. Thus, together with the finding of Sullivan *et al.* (1983), our results clearly suggest that the turnover rate in different cell types is at least involved in the regulation of GPDH activity during *Drosophila* development.

The isozymes of GPDH in *Drosophila* appear to be structurally similar. Genetic analysis indicates that each isozymes may be arised from the same structural gene. It seems then that the same or similar evolutionary adaptive response may be accomplished by either gene duplication or epigenetic mechanisms. Experimental evidence shown in this study strongly indicate that GPDH is a single structural gene. However, there are physicochemical differences: each isozymic form has a different heat stability, electrophoretic mobility and isoelectric point (pI). They also differed in tryptic digestion and finger print analysis. Anyway, the structural differences were little and it may be due to the addition or subtraction of a low-molecular-weight mobility (Bewley and Lucchesi, 1976; Bewley and Miller, 1979; Niesel *et al.*, 1980). Niesel *et al.* (1982) previously proposed that GPDH-3 was formed by a proteolytic cleavage of three amino acids from the carboxyl terminal end of GPDH-1. The present results appear to support a mechanism of epigenesis for the origin and differentiation of GPDH isozymes in *Drosophila melanogaster*. It is, however, also possible that the expression of each isozyme is a result of differential processing of nuclear RNA into cytoplasmic translatable mRNA. Such a mechanism has been demonstrated in mouse immunoglobin μ chain (Early *et al.*, 1980), mouse α-amylase (Young *et al.*, 1981), *Drosophila* myosin heavy chain (Rozek

and Davidson, 1983) and fibronectin (Schwarzbauer *et al.*, 1983). Indeed, further works such as the molecu'ar approach with cloned GPDH gene (Cook *et al.*, 1986) will provide more convincing evidence on the molecular mechanisms underlying the origin of GPDH isozymes.

References

Ashburner, M. and J. N. Tompson, Jr., 1978. The Laboratory Culture of *Drosophila*. *In*: The Genetics and Biology of *Drosophila* (M. Ashburner and T.R.F. Wright, eds.). Academic Press, London, Vol. 2a, pp. 2-81.

Bailey, G.C., 1984. The Production of Antisera. *In*: Method in Molecular Biology (J.M. Walker, ed.). Humana Press, Cliton, New Jersey, Vol. 1, pp. 295-300.

Bewley, G.C. and J.C. Lucchesi, 1976. Origin of α-glycerol-3-phosphate dehydrogenase in *Drosophila melanogaster* and their functional relationship in α-glycerphosphate cycle. *Biochem. Genet.* **15:** 235-252.

Bewley, G.C. and S. Miller, 1979. Origin and differentiation of the soluble α-glycerophosphate dehydrogenase isozymes in *Drosophila melanogaster*. *In*: Isozymes; Current Topics in Biological and Medical Research (M.C.Rattazi, J. G.Scandalios, and G. S. Whitt, eds.). Alan R. Liss, New York, Vol. 3, pp. 23-52.

Cook, J. L., J. B. Shaffer, G. C. Bewley, R. J. MacIntyre, and D. A. Wright, 1986. Isolation of a genomic clone for *Drosophila* sn-glycerol-3-phosphate dehydrogenase using synthetic oligonucleotides. *J. Biol. Chem.* **261:** 11751-11755.

Early, P., J. Roger, M. Davis, K. Calame, M. Bond, R. Wall, and L. Hood, 1980. Two mRNA can be produced from a single immunoglobulin μ gene by alternative RNA processing pathways. *Cell* **20:** 313-319.

Grell, E. H., 1967. Electrophoretic variants of α-glycerophosphate dehydrogenase in *Drosophila melanogaster*. *Science* **158:** 1319-1320.

Joweet, T., 1986. Preparation of nucleic acid. *In: Drosophila:* A Practical Approach (D. B. Roberts, ed.). Information Printing Ltd., Oxford, England, pp. 278.

Kim, S. J., C. C. Lee, and S. G. Paik, 1987. The effects of temperature on the developmental profiles of α-glycerol-3-phosphate dehydrogenase and lactate dehydrogenase during the development of *vestigial* mutant of *Drosophila melanogaster*. *Korean J. Genetics* **9:** 153-162.

Kotarski, M. A., S. Pickert, and R. J. MacIntyre, 1983. A cytogenetic analysis of the chromosomal region of *Drosophila melanogaster*. *Genetics* **105:** 371-386.

Laemmli, U. K., 1970. Cleasvage of structrual protein during the assembly of the head of bactriophage T_4. *Nature* **227:** 680-685.

Laskey, R. A. and A. D. Mills, 1975. Quantitative film detection of ^{3}H and ^{14}C in polyacrylamide gels by fluorography. *Eur. J. Biochem.* **56:** 335-341.

Laurell, C., 1966. Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies. *Anal. Biochem.* **15:** 45-52.

Lowry, O., A. Rosebrough, A. Farr, R. Randall, 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193:** 266-275.

McDonald, J.F. and J.C. Avise, 1976. Evidence of the adaptive significance of enzyme activity levels: Intraspecific variation in alpha-glycero-phosphate dehydrogenase in *Drosophila*. *Biochem. Genet.* **14:** 347-355.

Miller, S. and E. Berger, 1979. The purification of α-glycerophosphate dehydrogenase from *Drosophila melanogaster* larvae and partial structural comparison with the form purified from adults. *J. Insect Physiol.* **9:** 355-364.

Mitchell, H. K. and A. U. Mitchell, 1964. Mass culture and age selection in *Drosophila*. *Dros. Inf. Ser.* **39:** 135.

Niesel, D. W., G. C. Bewley, S. G. Miller, F. B. Amstrong, and C.-Y. Lee, 1980. Purification and structural analysis of the soluble sn-glycerol-3-phosphate dehydrogenase isozymes in *Drosophila melanogaster*. *J. Biol. Chem.* **255:** 4073-4080.

Niesel, D. W., Y.-C. E. Pan, G. C. Bewley, F. B. Amstrong, and S. S.-L. Li, 1982. Structural analysis of adult and larval isozymes of sn-glycerol-3-phosphate dehydrogenase of *Drosophila melangaster*. *J. Biol. Chem.* **257:** 979-983.

Oakley, B. R., D. R. Kirsh, and N. R. Morris, 1980. A simplified ultrasensitive silver stain for detecting proteins in polyacrylamide gels. *Anal. Biochem.* **105:** 361-363.

O'Brien, S. J. and R. J. MacIntyre, 1972a. The α-glycerophosphate cycle in *Drosophila melanogaster*. I. Biochemical and developmental aspects. *Biochem. Genet.* **7:** 141-161.

O'Brien, S. J. and R. J. MacIntyre, 1972b. The α-glycerophosphate cycle in *Drosophila melanogaster*. II. Genetic aspects. *Genetics* **71:** 127-138.

O'Farrell, P. H., 1975. High resolution two-dimensional electrophoresis of proteins. *J. Biol. Chem.* **250:** 4007-4021.

Rozek, C. E. and N. Davidson, 1983. *Drosophila* has

one myosin heavy-chain gene with three developmentally regulated transcripts. *Cell* **32:** 23-34.

Schwarzbauer, J. E., J. W. Tamkun, I. R. Lemischka, and R. O. Hynes, 1983. Three different fibronectin mRNA arise by alternative splicing within the coding region. *Cell* **35:** 421-431.

Skuse, C. R. and D. T. Sullivan, 1985., Developmentally regulated alternative modes of expression of *Gpdh* Locus of *Drosophila*. *EMBO. J.* **4:** 2275-2280.

Sullivan, D. T., F. A. Donovan, and G. R. Skuse, 1983. Developmental regulation of glycerol-3-phosphate dehydrogenase synthesis in *Drosophila*. *Biochem. Genet.* **21:** 49-62.

Towbin, H., T. Steahelim, and J. Gordon, 1979. Electrophoretic transfer of proteins from polyacrylamide gel to nitrocellulose sheets: Procedure and some application. *Proc. Natl. Acad. Sci.* USA **76:** 4350-4354.

Wilkins, J. R., J. B. Shaffer, and G. C. Bewley, 1982. *In vivo* radiolabeling and turnover of sn-glycerol-3-phosphate dehydrogenase in *Drosophila melanogaster:* Gene dosage effects. *Dev. Genet.* **3:** 129-142.

Winter, A., H. Perlmutter, and H. Davies, 1980. Preparative Flat-Bed Electrofocusing in granulated gel with LKB 2117 Multiphor. LKB Application Note 198.

Wright, D. A. and C. R. Shaw, 1969. Genetics and ontogeny of α-glycerophosphate dehydrogenase in *Drosophila melanogaster*. *Biochem. Genet.* **3:** 345-353.

Young, R. A., O. Hagenbuchle, and U. Schibler, 1981. A single mouse α-amylase gene specifies two different tissue specific mRNAs. *Cell* **23:** 451-458.

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노랑초파리의 α-Glycerol-3-phosphate Dehydrogenase (GPDH)의 발현과 조절

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노랑초파리의 α-glycerol-3-phosphate dehydrogenase (GPDH)의 발생단계 및 조직특이적 발현에 대한 발생유전학적 조절 양상을 연구하였다. 노랑초파리의 GPDH는 주로 흉부와 복부에서 각각 활성을 보이는 GPDH-1과 GPDH-3의 두가지 동위효소로 이루어져 있는데, 성충에서 총 GPDH 활성의 62%는 흉부에서 나타내며 복부에서는 총 GPDH 활성의 약 32%를 보였다. 그러나 두 조직에서 GPDH의 상대적인 합성량은 서로 비슷하여 GPDH-3가 GPDH-1보다 훨씬 빨리 turnover됨을 암시하였다. 순수분리한 GPDH-1으로 항혈청을 만든 후 두 동위효소의 면역학적인 동질성을 조사한 결과, 두 효소의 구조적인 차이는 매우 근소하였다. GPDH 동위효소의 기원을 규명하기 위해서 유충과 성체 조직으로 immunoblotting과 *in vitro* translation을 수행한 바, 단백질 수준에서 GPDH-1의 단일체는 GPDH-3의 단일체보다 분자량이 다소 큰 것으로 나타났다. 그러나 유충과 성체에서 추출한 RNA로 합성한 translation 산물을 분석한 결과, 분자량이 동일한 하나의 band만 검출되는 것으로 보아, GPDH-3는 GPDH-1에서 post-translational modification에 의해 생성되는 것으로 사료되었다.