The Polyadenylation State of α-glycerol-3-phosphate Dehydrogenase (GPDH) mRNA during development of *Drosophila melanogaster*

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노랑 초파리 발생단계에 따른 α-glycerol-3-phosphate dehydrogenase (GPDH) mRNA의 Polyadenylation 상태의 변화

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ABSTRACT

The polyadenlylation state of GPDH mRNA involved in abundant classes of RNAs were investigated using *in vitro* translation and two dimensional gel electrophoresis. These results argued that many mRNA which were predominantly poly(A)$^+$ in embryo become more evenly distributed between the poly(A)$^+$ and poly(A)$^-$ compartment during subsequent development. The polypeptide encoded by GPDH mRNA was involved in this case.

INTRODUCTION

The differential expression of a specific gene in both space and time is of fundamentally importance in elucidating the molecular aspects of eukayotic development. It has been however little known about the molecular mechanisms underlying developmental regulation of gene expression.

The concept of differential gene activity implies that cells which are genetically or developmentically different from one another should be characterized by a different constellation of proteins. In *Drosophila*, there are a large amount of information supporting the concept that the different imaginal discs are genetically distint from one another (Upsprung

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and Nothiger, 1972; Postlethwait and Schneiderman, 1974; Gehring, 1976; Fristrom, 1972).

Alpha-glycerol-3-phosphate dehydrogenase (GPDH, EC. 1.1.1.8) in *Drosophila melanogaster* represents an ideal system for the examination of gene expression during development (O'Brien and MacIntyre, 1972; Bewley and Miller, 1979; Kim *et al.*, 1987). Its activity is associated with three isozymic species (GPDH-1, GPDH-2, and GPDH-3) that are uniquely distributed with repect to developmental and tissue specificity. Intensive genetic and biochemical analyses suggest that the primary structure of each isozyme is nearly identical; a homodimer with subunit molecular weight of 31,700 daltons (Bewley and Miller, 1979; Miller and Berger, 1979; Niesel *et al.*, 1980). Considerable evidence exists that GPDH isozymes are the product of a single structural gene, and GPDH-3 may be arised by a specific post-translational modification of GPDH-1. However, it is possible that the expression of each isozyme is a result of a differential processing of nuclear RNA into cytoplasmic translatable mRNA.

Recent studies indicate that approximately two-third of polysomal RNA sequences complexity is contributed to a discrete class of mRNA existing solely in nonadenylated form at third instar larva, pupa, and adult of *Drosophila* development (Zimmerman *et al.*, 1980; Levy and Manning, 1981; Winkles and Grainger, 1985). However, these studies are rather limited since the properties of rare class mRNA are only concerned. In this respect, it seems to be necesary to investigate the polyadenylation state of abundant class mRNA as well. Since GPDH protein constitutes 1-2% of soluble proteins in *Drosophila*, this study was performed to investigate the polyadenylation state of GPDH mRNA during the entire developmental stages of *Drosophila melanogaster*.

MATERIALS AND METHODS

1) Drosophila culture

The Oregon-R wildtype stock of *Drosophila melanogaster* was maintained in uncrowded half-pint bottles on standard cornmeal-molasses-yeast-agar medium containing propionic acid as mold inhibitor. Cultures were supplemented with live yeast and maintained at 25℃. When needed, the flies were grown in mass culture at 25℃ and 65% humidity (Mitchell and Michell, 1964). The developmental stages of *Drosophila* was identified according to Ashburner and Tompson (1978).

2) Electrophoretic procedures

SDS-polyacrylamide gel electrophoresis was conducted utilizing the vertically oriented slabs. Running and stacking gels, electrode buffer, and SDS concentration have been described previously by Laemmli (1970).

Two dimensional gel electrophoresis was performed according to the procedure of O'Farrell (1975). For fluorography, the gels were impregnated with PPO, dried, and exposed to Kodak XR film, as described by Laskey and Mills (1975).

3) RNA extractions

Total RNA was prepared from embryo, 4-day-old larva, pupa, and adults synchronized at emergence according to Jowett (1988). The final precipitated RNA was removed by centrifugation, washed in 70%

ethanol. The pellet was washed with 2M LiCl in order to remove DNA, redissolved in 0.1 M sodium acetate (pH 7.0). RNA was reprecipitated by adding of 2.5 volume of absolute ethanol and stored at -70℃. The RNA concentration was determined by absorption at 260nm.

4) Oligo d(T)-cellulose chromatography

For poly(A)$^+$ RNA isolation, total RNA was passed through oligo d(T)-cellulose column (Daveis *et al.*, 1986). RNA sample dissolved in loading buffer A (20 mM Tris, pH 7.4, 0.5 mM NaCl, 0.5 mM EDTA, and 0.5% SDS) was heat-denatured, cooled, and applied to column; the flow-through RNA was reapplied three times. Poly(A)$^-$ RNA fraction was recovered by washing column with 5 ml of loading buffer B (20 mM Tris, pH 7.0, 0.1 M NaCl, 1 mM EDTA, and 0.1% SDS). Poly (A)$^+$ RNA was eluted with 1.5 ml of elution buffer (10 mM Tris, pH 7.4, 1 mM EDTA, and 0.05% SDS).

5) Rabbit reticulocyte cell-free translation

Translation was carried out in a reation volume of 13 μl of a rabbit reticulocyte translation system (Amersham) containing 1 μl of [^{35}S]-methionine (Amersham) and 2 μl of RNA accoridng to manufacturer's instructions. The translation mixture were incubated at 30℃ for 90 min.

RESULTS AND DISCUSSION

Total RNAs from individuals at particular developmental times (embryo, larva, pupa, and adult) were extracted, then translated *in vitro* in a message-dependent rabbit reticulocyte cell-free translation system. Figure 1 showed a linear response of translation with a amounts of RNA added and [^{35}S]-methionine incorporation.

The polyadenylation state of abundant classes of mRNA during *Drosophila* development was investigated using cell free translation method. Poly(A)$^+$ and poly(A)$^-$ RNA prepared from embryo, larva, pupa, and adult were used. The translation products of all four stages were electrophoresed on two dimensional polyacrylamide gels (Figure 2). Although approximately 150 polypeptides were synthesized in the poly(A)$^+$ RNA translation, only a few products synthesized were stage specific. The relative intensity of many common polypeptides in poly(A)$^-$ RNA translation appeared to be higher in larva, pupa, and adult than embryo.

Although GPDH mRNAs of embryo stage were detected in poly(A)$^+$ RNA compartment, they became evenly distributed between compartments of poly(A)$^+$ and poly(A)$^-$ during subsequent development (Figure 2, see arrows). Finally, when the poly(A)$^+$ and poly (A)$^-$ RNA translation products from developmental stages were compared each other, poly(A)$^-$ mRNA sequences were ordinally involved in poly(A)$^+$ mRNA compartment. Thus, it was apparent that poly (A)$^-$ mRNA sequences represented a subset of the poly(A)$^+$ mRNA population.

Most *Drosophila* embryonic non-histone mRNA were enriched in poly(A)$^+$ class of RNA. This result is compatible with that of Winkles and Grainger (1985), but contrast to the demonstration in sea urchin embryo; a similar *in vitro* translation analysis indicates that most abun-

dant mRNA sequences in these embryo coexisted at similar concentration in both the poly (A)$^+$ and poly(A)$^-$ RNA compartments (Brandhorst *et al.*, 1979). During postembryonic development, GPDH mRNA become more evenly distributed between the poly(A)$^+$ and poly(A)$^-$ classes.

Zimmerman *et al.* (1980) and Levy and Manning (1981) found that a large number of mRNA sequences are restricted to the poly(A)$^-$ RNA class in third instat larva, pupa, and adult. Their experiments, employing RNA-excess hybridization to single-copy DNA, predominantly reflected the properties of mRNA present in a rare frequency class.

This studies using *in vitro* translation analysis examined the distribution of poly (A)$^+$ tracts within a more abundant mRNA population. In agreement with others (Burckhardt and Brirnstiel, 1978; Spradling *et al.*, 1979; Winkles and Grainger, 1985), the embryonic histone mRNA species was predominantly poly(A)$^-$. Any non-histone mRNA species which exist solely as poly(A)$^-$ mRNA was not detected during *Drosophila* development. This results suggest that in *Drosophila*, with a exception of histone mRNA, rare class mRNA were predominantly nonadenylated, and abundant mRNA were predominantly polyadenylated (embryonic RNA) or bimorphic (postembryonic RNAs). A similar relationship may exist in sea urchin eggs; Duncan and Humphreys (1981) had found that although both rare and abundant egg mRNA were bimorphic, approximately two-thirds of the rare class sequenc exist predominantly as poly(A)$^-$ RNA. Therfore, the concentration of individual mRNA may be influenced by their 3'-terminal sequence. In addition, since it was observed that abundant mRNA sequences were generally more stable than rarer mRNA (Lenk *et al.*, 1978; Meyuhas and Perry, 1979), poly(A) may exert their influence on mRNA concentration by conferring stability on poly(A)$^+$ RNA sequences.

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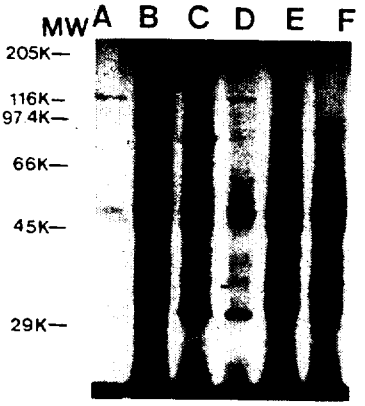

Figure 1. One dimensional gel analysis of *in vitro* translation products synthesized by total RNA added to a rabbit reticulocyte system. Total RNA translation products of a particular developmental time were electrophoresed on 10% SDS-polyacrylamide gel. The radiolabeled polypeptides were detected by fluorography. (A) No RNA added, (B) 6 μg of embryonic RNA, (C) 4 μg of third-instar larval RNA, (D) 3 μg of pupal RNA, (E) 7 μg of 5-old-days adult RNA.

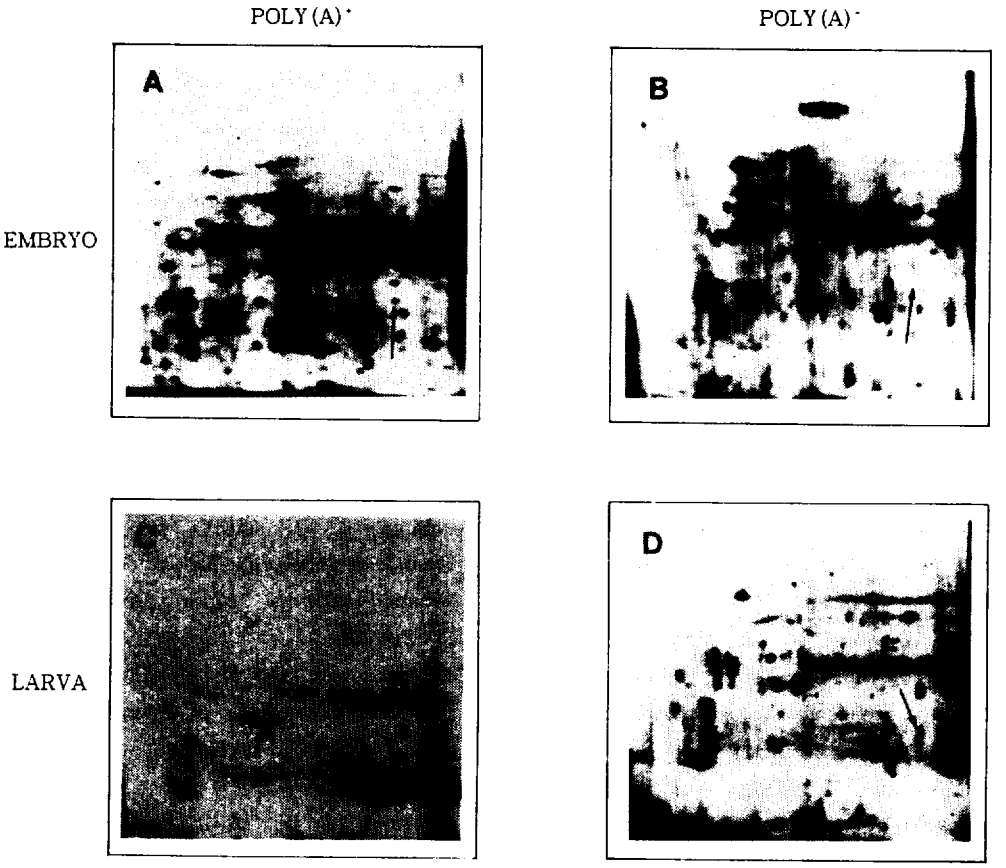

Figure 2. Two-dimensional gel analysis of translation products synthesized by poly(A)$^+$ and poly(A)$^-$ RNA prepared from different stages of the *Drosophila* life cycle. Poly(A)$^+$ and poly(A)$^-$ RNA were prepared from embryo, larva, pupa, and adult. Ten μg of poly(A)$^-$ or 0.2 μg of poly(A)$^+$ RNA was translated in the rabbit reticulocyte system. The translation products were electrophoresed on pH 5-7 isoelectric focusing tube gels and then 10% SDS slab gels. Second dimension electrophoresis was carried out from top to bottom, with the basic end of the pH gradient at the right side of each gel. Radiolabeled polypeptides were detected by flurography. (A) Embryo, poly (A)$^+$ RNA; (B) embryo, poly(A)$^-$ RNA; (C) larva, poly(A)$^+$ RNA; (D) larva, poly(A)$^-$ RNA; (E) pupa, poly(A)$^+$ RNA; (F) pupa, poly(A)$^-$ RNA; (G) 5-old-days adult, poly(A)$^+$ RNA; (H) 5-old-days adult, poly(A)$^-$ RNA. A, actin; E, radiolabeled polypeptides which was endogenous to the rabbit reticulocyte system. The arrows donated the position of GPDH subunit synthesized by stage specific GPHD mRNA.

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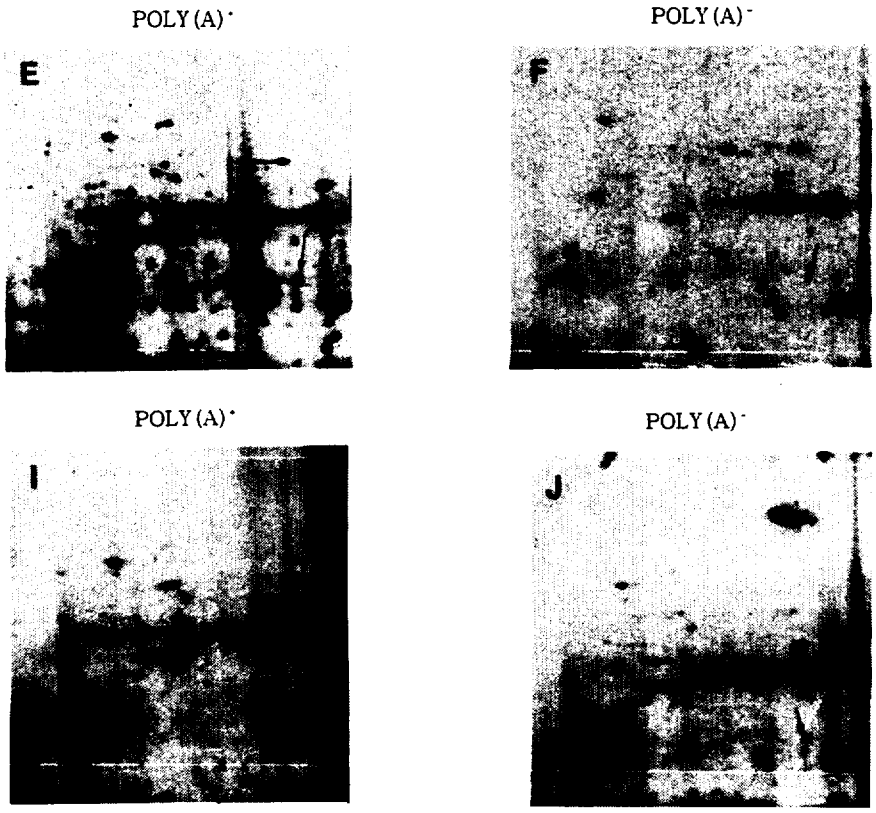

摘　　要

노랑초파리(*Drosophila melanogaster*)에서 α-glycerol-3-phosphate dehydrogenase(GPHD)의 mRNA는 풍부한 군(abundant mRNA)에 속한다. 따라서 발생단계에 따른 GPDH mRNA의 polyadenylation 상태의 변화를 *in vitro* translation과 2차원 전기영동 방법으로 분석하였다. Embryo 시기에 poly(A)$^+$군에 속하던 많은 mRNA들이 점차 발생이 진전됨에 따라 poly(A)$^+$와 poly(A)$^-$군으로 균등하게 분포되는 양상을 보였는데, GPDH mRNA도 이런군에 포함되었다.