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Structural and Functional Evolution of Class I Aldolase Gene

Tatsuo Kai

<Abstract>

FBP aldolase (EC 4.1.2.13) is a glycolytic enzyme widely distributed in almost all organisms. It catalyzes reversibly the fission of a hexose bis- or monophosphate, *i.e.*, FBP or F1P, yielding dihydroxya cetonephosphate and glyceraldehyde 3-phosphate or glyceraldehyde. Regarding the enzyme as a suitable model of isozyme systems, numerous works have been done for elucidating the molecular mechanisms of enzymatic function as well as for understanding the evolutional differentiation of isozymes from the standpoint of comparative biology. For the same purpose, the gene structures and mRNA expression of aldolases has been well investigated. This article was devoted to a review on the hitherto established knowledge about aldolases: classification of aldolases from general sources, characteristics of vertebrate aldolases with special reference to gene structure and expression, and finally some aspects of the *D. melanogaster* aldolase, giving some notion on the gene evolution by comparing vertebrates and the insect.

Key words: aldolase, class I, gene, evolution

Class I and Class II aldolases

Rutter (1964) divided FBP aldolases into two classes (Class I and Class II). Class I aldolases were found in animals, higher plants, and some prokaryotic species. These are tetrameric proteins which form a Schiff base as reaction intermediate and is, thus, active in the absence of divalent ions. It has a native molecular weight of 160,000 composed of four identical subunits each of which is 40,000 (Kawahara and Tanford, 1966). Sequence studies of a number of vertebrate class I aldolases have led to the identification of the substrate-binding lysyl residue and have uncovered extensive structural homology in the adjacent positions of the polypeptide chain (active site peptide) (Lai and Oshima, 1971; Lai and Chen 1971; Gibbons *et al.*, 1972; Ting *et al.*, 1971a, 1971b; Forcina and Perham, 1971; Morse and Horecker, 1968; Mareschal *et al.*, 1975; Lee *et al.* 1975; Guha *et al.*, 1971). The position of active-center lysyl residue was confirmed by isotope labeling with $NaBH_4$ reduction under the presence of a substrate (Lai and Oshima, 1971; Ting *et al.*, 1971a, 1971b).

Class II aldolases were found in fungi (Rutter, 1964) and in most prokaryotes including all cyanobacteria as far as investigated so far. Class II aldolases are dimeric proteins which form an endiole as reaction intermediate and depend on divalent ions and therefore are inhibited in the presence of EDTA. A metal ion functions as an electrophilic center for substrate polarization. It has a native molecular weight of 80,000 composed of a subunit of 38,000. There is no significant sequence homology between the two classes of aldolase. In terms of evolution, this implies

* Departent of Nutritional Sciences, Senan Jo Gakuin University, 1-3-5, Ibori, Kokurakita-ku, Kitakyushu-shi, Fukuoka 803-0835, Japan

that class II aldolase cannot be the progenitor of class I aldolase nor *vice versa*. Interesting facts are that *E.coli* contains both a class I and a class II aldolase (Stribling and Perham, 1973) and that *Euglena gracilis* contains a plastidic class I and a cytosolic class II aldolase (Mo *et al.*, 1973). The plastid and cytosol aldolase of plants are well investigated and characterized for the evolutional differentiation between the two classes of aldolase (Anderson and Advani, 1970; Kruger and Schnarrenberger, 1983; Lebherz *et al.*, 1984a, 1984b; Schnarrenberger, 1987; Kelly and Tolan, 1986; Hidaka *et al.*, 1990; Flechner *et al.*, 1999; Gross *et al.*, 1999).

Enzyatic properties of aldolase isozymes from vertebrates

Vertebrate aldolases have three distinct isozymes A, B, and C which show tissue-specific distribution (Lebhartz and Rutter, 1969; Penhoet *et al.*, 1966). Aldolase A, which prefers FBP as substrate to F1P, exists predominantly in the muscle as well as in the brain and certain other tissues. The activity ratio of FBP/F1P of this isozyme is 50. Aldolase B, which utilizes equally FBP and F1P, and hence whose FBP/F1P ratio is 1, is present mainly in the adult liver. Aldolase C, the third isozyme, is brain-specific and has an FBP/F1P ratio of 10. Aldolase A is involved primarily in glycolysis taking place in the muscle. On the other hand, aldolase B plays in the liver a major role in gluconeogenesis and in the catabolism of F1P, the major intermediate involved in the breakdown of dietary fructose. Aldolase C functions both in glycolysis and glycogenesis in the brain. These isozymes change their distribution patterns during development. In early embryonic stages aldolase A appears predominantly in the liver along with a small amount of aldolases B and C, but before birth aldolases A and C progressively diminish, while aldolase B begins to rise in amount and becomes a major form in the adult liver (Rutter *et al.*, 1963; Matsushima *et al.*, 1968). In *Xenopus laevis*, mRNA expression of aldolase isozymes are well studied (Shiokawa *et al.*, 2002). That is, aldolase A and C mRNAs are major aldolase mRNAs in early stges of *Xenopus* embryogenesis which proceeds utilizing yolk as the only energy source. Aldolase B mRNA, on the other hand, is expressed only later in development in tissues which require aldolase B for dietary fructose metabolism. The resurgence of a fetal isozyme in cancer was often observed (Schapira, 1981). Aldolase A reappeared in a fast growing hepatoma with concomitant disappearance of aldolase B that is abundant in the normal adult liver (Gracy *et al.*, 1970; Horecker *et al.*, 1972; Schapira, 1981).

Amino acid sequences of aldolases

Comparison of amino acid sequences of vertebrate aldolase isozymes reveals that the proteins are composed of the sequences with three different characteristics: CCS (common conserved sequence), IGS (isozyme group specific), and divergent sequences (Kitajima *et al.*, 1990; Takasaki and Hori, 1990). These features are shown in Figure 1. CCSs are the major constituents of eight-fold TIM-barrel structure (see below) in which the active site of the enzyme is located (Kitajima *et al.*, 1990; Takasaki and Hori, 1990). The IGSs are located at three sites on the amino-terminal region (IGS-1, 2, and 3) and at a site on the carboxyl-terminal region (IGS-4) of the enzymes (Kitajima *et al.*, 1990). The carboxyl-terminal region bearing the Tyr^{363} and the amino-terminal region spanning amino acid residues 34-108 were required to determine the characteristics as isozyme A: a high catalytic activity toward FBP and a low catalytic activity toward F1P, thus a high FBP/F1P ratio (Kitajima *et al.*, 1990; Takasaki and Hori, 1990). The significance of the carboxyl-terminal region in determining the characteristics of isozyme A was demonstrated by several different studies such as chimeric enzyme construction (Kitajima *et al.*, 1990; Takasaki and Hori, 1990, 1992), enzyme modifications (Drechsler *et al.*, 1959; Rutter *et al.*, 1961), and site-directed mutagenesis (Takasaki *et al.*, 1990a, 1990b). Recent study revealed that IGS-1 in concert with IGS-4 are

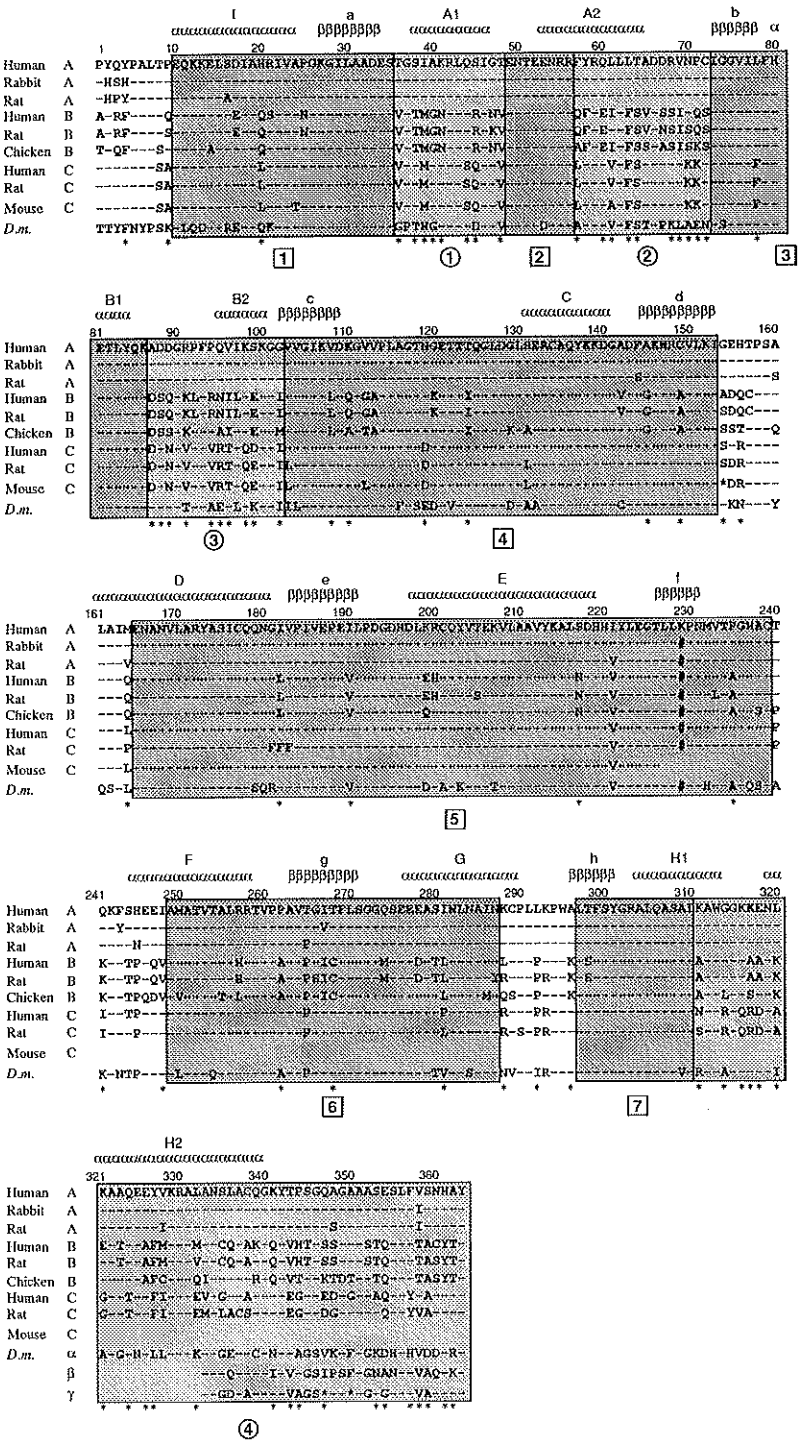

Figure 1. Amino acid sequences of Class I aldolases (A, B, and C). Amino acids identical to the human aldolase A sequence are expressed by dashes. Regions residing in commonly conserved sequence (CCS) are boxed and darkly shaded (boxed numbers 1-7). Similarly, isozyme group specific (IGS) residues are marked by asterisks, boxed and lightly shaded (circled numbers 1-4). The active-site Lys[229] is indicated (#) in the sequence frame. Regions in α-helix (ααα-αα, I, A1-H2) and β-sheet (βββ-ββ, a-h) structures of rabbit aldolases A (Sygusch *et al.*, 1987) are diagrammed over the sequence. Literature sources are as follows: Human aldolase A (Sakakibara *et al.*, 1985a), human aldolase B (Sakakibara *et al.*, 1985b), human aldolase C (Rottmann *et al.*, 1987), rat aldolase A (Joh *et al.*, 1985), rat aldolase B (Tsutsumi *et al.*, 1985), rat aldolase C (Kukita *et al.*, 1988), rabbit aldolase A (Tolan *et al.*, 1984), chicken aldolase B (Burgess and Penhoet, 1985) and mouse aldolase C (Paolella *et al.*, 1986).

mainly responsible to exhibit the characteristics as isozyme A. Moreover, amino acid residues at positions 41 and 45, both of which are isozyme-specific, possibly have an important role in determining the catalytic activity of aldolase isozyme (Motoki *et al.*, 1993).

Role of C-terminal region

The three-dimensional structures of rabbit muscle aldolase and human muscle aldolase have been determined by X-ray crystallography at 2.7 and 3.0 Å resolution (Sygusch *et al.*, 1987; Gamblin *et al.*, 1990, 1991), respectively. The basic feature of the FBP aldolase subunit is the eight-stranded α/β-barrel (TIM-barrel) found also in other glycolytic enzymes (Alber *et al.*, 1981; Stuart *et al.*, 1979; Mavridis *et al.*, 1982). The conformation at the carboxyl-terminal region has not been well-known, but it was revealed that the carboxyl-terminal arm is flexible and mobile and is possibly responsible for the opening and closing of the active site cleft during the catalytic process (Hester *et al.*, 1991).

Gene structure of aldolase isozymes

Genes for all the three isozymic forms of aldolases A, B, and C have been cloned from the rat and human (Joh *et al.*, 1986; Mukai *et al.*, 1987, 1991a; Maire *et al.*, 1987; Tsutsumi *et al.*, 1985; Rottman *et al.*, 1987), the B gene from the chicken (Burgess and Penhoet, 1985) and the C gene from *Xenopus laevis* (Yatsuki *et al.*, 1998). As for the protein-coding exons, all these genes have essentially the same structure; the coding sequences are interrupted by the introns at the same sites in all of the aldolase genes analyzed (Figure 2). The genes encoding these subunits are not identical and are assumed to be located on the separate chromosomes (Penhoet *et al.*, 1967; Benfield *et al.*, 1979; Lai, 1975). The genes for the

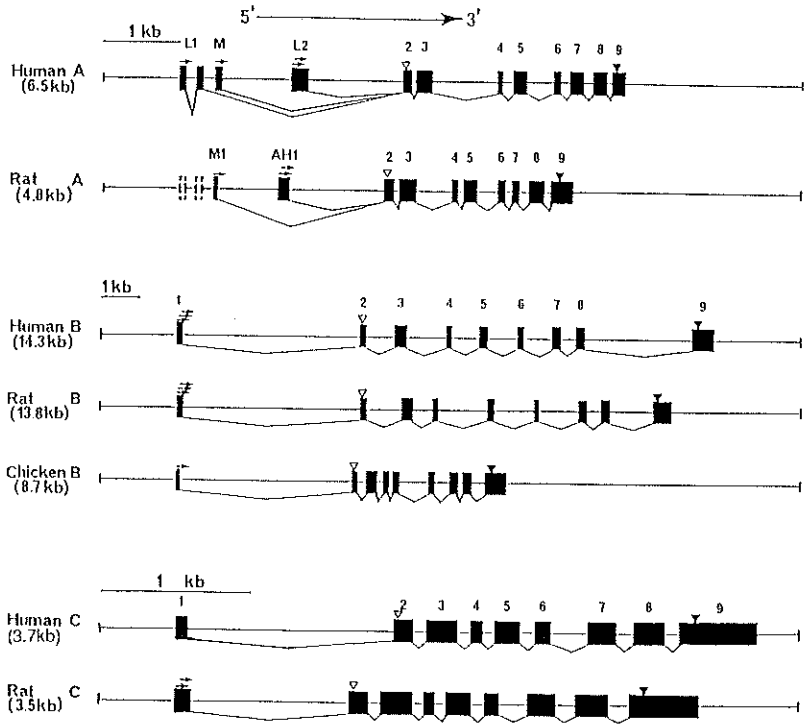

Figure 2. Structures of aldolase isozyme genes in vertebrates (Joh *et al.*, 1990). Scales for drawing are different among genes for different forms. Size of the gene is shown below the name of the gene. Solid boxes indicate exons. Arrows above leader exons represent the respective transcription initiation codons. Splicing patterns are indicated with lines between adjacent exons. Open and closed triangles show the positions of translational initiation site and termination site, respectively. In rat aldolase A gene, broken boxes represent the regions homologous to human L1 exons.

human aldolases A, B and C have been mapped to the chromosomes #16 (Kukita *et al.*, 1987), #9 (Henry *et al.*, 1985) and #17 (Tolan *et al.*, 1985), respectively. *Xenopus* aldolase genes are localized on different chromosomes as a single copy gene (Shiokawa *et al.*, 2002). These facts indicate that these genes are derived from a common ancestral gene via polyploidization (Ohno, 1973; Ohno *et al.*, 1986).

mRNA expression of aldolase genes

The expression of these genes show multiple patterns and seems to be regulated independently; the three kinds of subunits are synthesized not simultaneously within the same cell or tissue but one or two of them are expressed. However, in some instances a manner of mutual influence is suggested: (1) Fetal cells and hepatoma cells sometimes contain all the three isozymes (Lebhertz and Rutter, 1969; Penhoet *et al.*, 1966; Lebherz, 1975), and (2) the increase in concentration of a particular type during development or carcinogenesis is often accompanied by an decrease in level of another pre-existing type; *e.g.*, the levels of the A and B types in the liver change reciprocally during development or hepatocarcinogenesis (Schapira *et al.*, 1963, 1975; Ikehara *et al.*, 1970; Numazaki *et al.*, 1984). The study of these closely related aldolase genes is of interest, with respect to the problem whether the control of expression is related to their specific structures, especially in the regions regulating transcription, and whether the expression of these genes is regulated in different ways.

The aldolase A genes (of the human and rat) differ from the aldolase B and C genes in the number of exons which encode the 5'-untranslated region of mRNA. The former has multiple exons, three for the human (exons L1, M, and L2) and two for the rat (exons M1 and AH1) (Mukai *et al.*, 1991a), whereas the latter has a single exon. Furthermore, the lengths of the corresponding introns of these genes are quite different (Hori *et al.*, 1987). Exons M1 and AH1 of the rat aldolase A gene correspond to exons M and L2 of the human gene, respectively. Although an homologous sequence to exon L1 of the human gene was found in the upstream region of the rat gene, mRNA with this sequence was not detected in the rat (Mukai *et al.*, 1991b). These leader exons are selectively used for gene expression in different tissues (Mukai *et al.*, 1986). In consequence multiple mRNAs with different 5'-untranslated sequences are expressed from a single gene (see the arrows in Figure 1). These mRNAs share a common protein coding sequence. Aldolase B and C genes so far examined have the same exon-intron organization (9 exons and 8 introns), while these genes from the human, rat, and chicken are different in size. The difference is due to the length of the corresponding introns. The first exon of the rat and human aldolase B genes and the rat aldolase C gene generates multiple mRNA species which start from closely located but different sites on the first exon (Arai *et al.*, 1989).

Although the significance of multiple promoters of the aldolase A genes is not yet understood, one possible explanation is that the rat M1 promoter had been created after diversion to fulfill a demand of skeletal muscle for high glycolytic activity levels (Joh *et al.*, 1990). Two liver-specific DNase I hypersensitive sites were detected in the rat aldolase B gene and related to transcription (Gregori *et al.*, 1991). One located just upstream from the cap site (weak core promoter), the second in the middle of the first, 4.8-kbp-long intron (strong activator). The L1 and L2 type mRNAs of the human A gene are rich in human hepatoma biopsies, although barely detectable in the normal liver (Gautron *et al.*, 1991).

D. melanogaster aldolase

Aldolase of *D. melanogaster* belongs to class I. Thus the insect's aldolase has been targeted to investigate to get a clue to the evolutional differentiation flow of class I aldolase gene by the comparative study with vertebrate. The

pupal aldolase from *D. melanogaster* has been well characterized previously, showing close resemblance to vertebrate aldolases (Brenner-Holzach and Zumsteg, 1982; Brenner-Holzach, 1979a, 1979b; Lai and Horecker, 1972). The complete amino acid sequence of the pupal aldolase was already determined (Malek *et al.*, 1985). It is a tetrameric enzyme containing four identical subunits of 360 amino acid residues with a molecular weight of 158,000. The aminoterminus is blocked by an acetyl group and terminal tyrosine is essential for the enzymatic activity toward FBP like other class 1 aldolases (Brenner-Holzach and Zumsteg, 1982; Brenner-Holzach, 1979a, 1979b; Malek *et al.*, 1985). Active-center lysine was identified for pupal aldolase (Brenner-Holzach and Zumsteg, 1982). The pupal aldolase has a broad range of optimum pH between 6.8-8.0. The K_m values for both of the substrates FBP (2.7×10^{-5}M) and F1P (1.85×10^{-2} M) closely resemble to those of the rabbit muscle enzyme (Brenner-Holzach and Leuthardt, 1967), but the FBP/F1P activity ratio (2,700/200=13.5) (Brenner-Holzach and Leuthardt, 1969) at substrate saturation is considerably lower than that of rabbit muscle aldolase.

As shown in Figure 1, the amino acid sequence of the pupal aldolase has strong homology to the rabbit muscle aldolase (71% identity) (Malek *et al.*, 1985) and shows essentially the same secondary-structure prediction as the vertebrate enzymes with one notable exception of the strongly predicted helix at residues 90-100 (Sawyer *et al.*, 1988). The core structure of *D. melanogaster* aldolase is essentially identical to those of the vertebrate aldolases and also has TIM-barrel structure (Brenner-Holzach and Smit, 1982; Hester *et al.*, 1991). The pupal aldolase subunits could form stable hybrid quarternary structures with subunits of certain mammalian aldolases (Brenner-Holzach and Leuthardt, 1972), indicating that the pupal enzyme had a similar spacial structure as those of mammalian enzymes. On the other hand, there are substantial differences in amino acid composition. The *D. melanogaster* aldolase contains only half the number of cysteinyl and histidyl residues present in the rabbit muscle aldolase (Brenner-Holzach, 1979a). Moreover, comparative studies of SH peptides have shown (Brenner-Holzach, 1979a, 1979b) that the insect aldolase does not have the two cysteinyl residues, one of which is suggested to be essential for enzyme function in the rabbit muscle aldolase (Cys^{72} or Cys^{336}). This finding highlighted the usefulness of further comparative sequence analysis for delineating the amino acid side chains essential for the class I aldolase catalysis (Brenner-Holzach and Zumsteg, 1982). Using the numbering employed for rabbit muscle aldolase by Tolan *et al* (1984), the *D. melanogaster* aldolase lacks a residue in positions 239, 334 and 353. There are also differences in total number of acidic amino acid residues. While both polypeptide chains carry nearly the same number of strongly basic residues (K+R=42 for the *D. melanogaster* pupal aldolase, K+R=41 for the rabbit muscle aldolase), the *D. melanogaster* aldolase contains more acidic residues (D+E=42) than the rabbit muscle aldolase (D+E=38), in agreement with the lower isoelectric point of the insect enzyme.

An aldolase cDNA was cloned from the cDNA library constructed from the adults of *D. melanogaster* (Kusakabe, 1990; Sugimoto *et al.*, 1990) and the cDNA was probed to clone the genomic DNA (Kai *et al.*, 1992). The aldolase gene from *D. melanogaster* was found to have an structure from which three distinct mRNAs could be generated by alternative splicing of the same primary transcript (Kai *et al.*, 1992; Shaw-Lee *et al.*, 1992; Kim *et al.*, 1992). The gene has three last exons, the selection of which can give rise to three isozyme forms differing at the carboxyl-terminal sequence. The schematic view of the gene structre is described in Figure 3. The entire aldolase gene except for 5'-terminal 8 bases of exon 2 and exon(s) 1 which is the noncoding exon(s). The coding region starting from exon 2 was 3.8 kb in length and had 5 exons (2, 3, 4α, 4β, and 4γ) separated by 4 introns. The exons 4α,

4β, and 4γ were similar to each other but non-identical in nucleotide sequence. These cognate exons are capable to encode three different carboxyl-terminal amino acid sequences (29, 31, and 31 residues, respectively) of aldolase. *D. melanogaster* aldolase gene is therefore predicted to generate three isozymes (referred to as α-, β-, and γ-types) from the same primary transcript by alternative splicing of the three final exons 4α, 4β, and 4γ. The deduced amino acid sequence of the γ-type aldolase (360 amino acids) corresponds completely to the reported sequence of the pupal enzyme (Malek *et al.*, 1985). In particular, the occurrence of two novel type mRNA species which retain two of the three last exons unspliced were confirmed. All of the five mRNAs, including those of the usual types and the novel types, were shown to occur at different developmental stages and the tissue specific expression was also found in the adult flies. The recombinant aldolase isozymes expressed in *E. coli* were functionally active and differed in enzymatic properties from each other (Kai *et al.*, 1992; Sugimoto *et al.*, 1995; Kai *et al.*, 1991a, 1991b).

Evolutional differentiation of the aldolase gene comparing vertebrates with D. melanogaster

In the *D. melanogaster* aldolase gene, exon 2 would be formed during evolution by fusion of the corresponding multiple exons (2 to 7 of

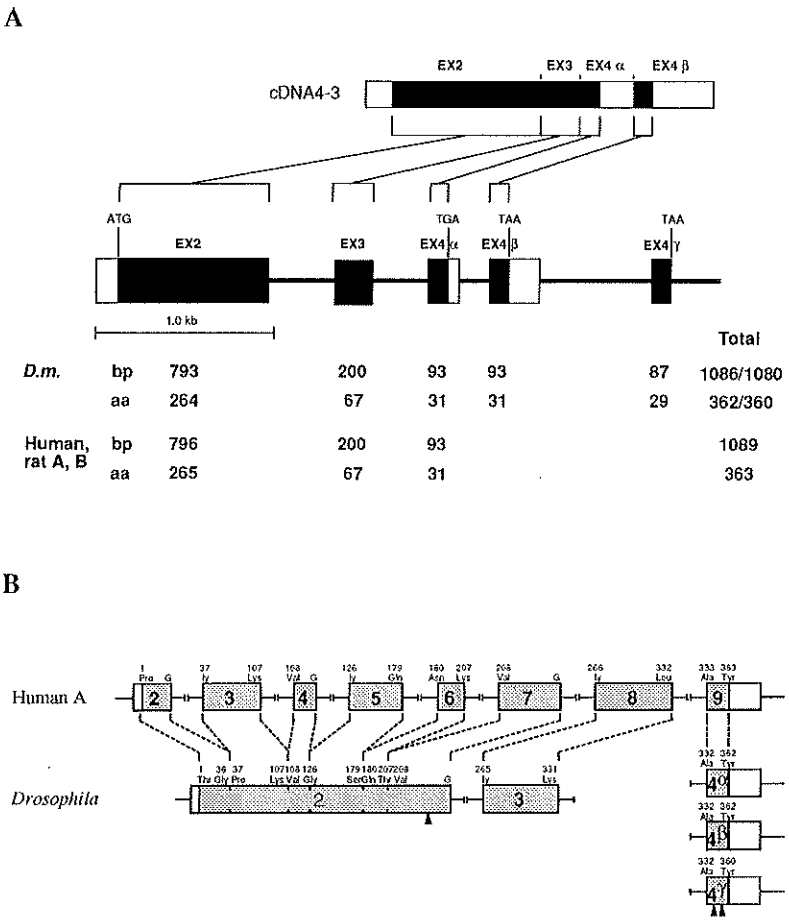

Figure 3. A: The *D. melanogaster* aldolase gene structure in comparison with the cDNA 4-3 structure (Sugimoto *et al.*, 1995). Open and closed boxes indicate noncoding and coding sequences, respectively. The number of amino acid residues encoded by each exon is indicated under the base pair number, both compared with corresponding numbers from human and rat aldolases A and B. Methionine residue encoded by the initiation codon (ATG) is not included in the numbers. B: Comparison of the exon-intron structures of the aldolase genes from *D. melanogaster* and the human. Shaded regions represent translated sequences. Single horizontal lines represent introns. Position of closed triangle (s) indicate an amino acid deletion.

the mammals). On the other hand, the exons 4α, 4β, and 4γ would be formed through tandem duplication of a single ancestral exon on the same chromosome. This is again in contrast to the mammals, where the three genes for the aldolase isozymes A, B, and C are located on different chromosomes, a fact which can be explained by assuming the occurrence of twice tetraploidization processes during evolution.

As was shown by reverse transcriptase PCR assay (Kai *et al.*, 1992), the presence of α-, β-, and γ-mRNA species, which were predicted to be generated through one of the three 3'-terminal exons (4α, 4β, and 4γ), strongly supports the idea that the alternative splicing event actually takes place *in vivo*. The sn-glycerol-3-phosphate dehydrogenase gene of *D. melanogaster* was also shown to generate three isozymes from the same transcript by alternative usage of exons 6, 7, and 8 (Cook *et al.*, 1988; Bewley and Cook, 1990). Similarly to the case of aldolase gene, exons 6, 7, and 8 has its own poly(A) addition site and each functions as final exon, generating proteins with different numbers of amino acid residues. This is a manner classified into a type of alternative polyadenylation sites. To the author's knowledge, aldolase and sn-glycerol-3-phosphate dehydrogenase of *D. melanogaster* provide sole examples in which the type-7 alternative splicing give mature mRNAs differing in sequence at coding regions. In both cases, each transcript and its cognate isozyme are at least to some extent stage- and tissue-specific in expression. Regulatory factors relating to alternative splicing and to polyadenylation must be linked or associated closely.

Northern hybridization analysis showed that aldolase mRNAs are expressed at various developmental stages. The gene expression at the larval and adult stages was much stronger than at the embryonic and pupal stages. This fact might be accounted for by the following assumptions. The aldolase is highly expressed and utilized in glycolysis to supply much energy for dietary behavior and active movements of the insects at the larval and adult stages, respectively. In contrast, gluconeogenesis requires rather moderate aldolase expression at the embryonic and pupal stages which are closed systems in terms of nutrition.

Reverse transcriptase PCR assay revealed the occurrence of two novel forms of mRNA for the *D. melanogaster* aldolase *in vivo*: αβ- and βγ-mRNAs. The αβ-mRNA corresponds to cDNA clone 4-3, whose precise structure was analyzed first by Kusakabe (1990) and Sugimoto *et al.* (1990). The presence of αβ-mRNA was also predicted, although ambiguously, by the two groups (Shaw-Lee *et al.*, 1992; Kim *et al.*, 1992). The βγ-mRNA is a novel finding in the present study. There is no direct evidence to show that the αβ-type and βγ-type mRNAs are functional in *D. melanogaster* tissues. However, the *E. coli* expression plasmid of cDNA clone 4-3, which derived from the αβ-type mRNA, was shown to be functional, generating the active isozyme in the host cells. The enzyme was found to have similar mobility on Zymograph to the recombinant α-type isozyme engendered from the pDAα expression plasmid. The expression plasmid for the βγ-mRNA remains to be constructed. Since the αβ-mRNA was strongly expressed in the adult abdomens, and the βγ-mRNA was expressed in the adult head and to some extent at the pupal stage, isozymes corresponding to these novel type mRNA species could in part be produced under certain physiological conditions in the adult flies, although definite conclusion will be drawn after additional experiments.

Multiple types of poly(A) signal may be present (Birnstiel *et al.*, 1985) but the typical one, AAUAAA works most efficiently than the other untypical ones in cleaving and polyadenylation during mRNA maturation (Sheets *et al.*, 1990; Proudfoot, 1991). Thus the presumed poly(A) signals of exons 4α (AUUAAU) and 4β (AAUAUA) would be used less efficiently in the gene expression than the typical poly(A) signal seen in exon 4γ. The lower efficiency is probably involved in the cause of the skipping of

signal function, thus producing the novel types of mRNAs with duplicated final exons.

As the γ-type isozyme appears to be the major component of the isozymes and is abundant in the insect tissues throughout development, it is likely that the γ-type mRNA is produced efficiently and serves as the major mRNA species to generate the enzyme. Since the mRNA for the α-isozyme was expressed in all the developmental stages and in all of the adult tissues tested, the enzyme appears to be ubiquitous, like vertebrate aldolase A (Mukai *et al.*, 1986). On the other hand, the mRNA for the β-isozyme was mainly expressed in the adult abdominal region, although it seems to be expressed slightly in other parts of the body and at earlier developmental stages, indicating that this isozyme plays a major role in the tissue-specific glycolytic metabolism in the abdominal region of adult flies. At present, there is no direct evidence that the γ-isozyme functions in nerve tissues like vertebrate aldolase C, but it is noteworthy that the mRNA for the γ-isozyme is mainly expressed in the heads and partly in the thoraxes of adult flies.

The γ-type isozyme has been found at the pupal stage as a tetrameric enzyme with four identical subunits (Malek *et al.*, 1985). Analyses of gene structure and expression of *D. melanogaster* aldolase strongly suggest that three different isozymic forms, α-, β-, and γ-types, of the insect aldolase should exist, although the α- and β-isozymes have not been isolated and characterized yet. The revealed characteristics of recombinant α-, β-, and γ-isozymes produced in *E. coli* host cells indicated that these isozymes are enzymatically active. Furthermore, these isozymes exhibit different catalytic activities toward the substrates (FBP and F1P) and the β-isozyme exhibited the strongest activities to both of the substrates.

The recombinant aldolase γ showed different specific activity both to FBP and F1P from the natural aldolase γ which is the only aldolase purified and characterized at present. Thus, certain modification on the γ-isozyme must be occurring *in vivo*. This situation is in quite contrast to the human aldolases where the natural enzymes and artificially expressed enzymes exhibited the same characteristics (Sakakibara *et al.*, 1989; Takahashi *et al.*, 1989; Kitajima *et al.*, 1990). It is of interest to isolate the natural α- and β-isozymes and characterize their enzymatic properties in comparison with those of the expressed isozymes. This may serve as an approach to solve the activation mechanism probably needed to the nascent aldolases.

The insect isozymes share short stretches with different amino acids at the carboxyl-terminal regions. In addition, the hydrophathy profile of the three isozymes are significantly different in the stretches at positions 334-340 and 347-359. Another interesting feature is the diversity of amino acid residues in these regions, particularly in the positions 347-359. Therefore, the three isozymes of *D. melanogaster* seem to be destined to have different general properties only by receiving which of the stretches at the carboxyl-terminal region. Possibly, the three isozymes of *D. melanogaster* aldolase take part in developmental stage- and/or tissue-specific sugar-phosphate metabolisms.

References

Alber T., Banner D.W., Bloomer A.C., Petsko G.A., Philips D.C., Rivers P.S., and Wilson I.A. (1981) *Phil. Trans. Roy. Soc. Lond.* **B.293**, 159-171.

Anderson L.E. and Advani V.R. (1970) *Plant Physiol.* **145**, 583-585.

Arai Y., Mukai T., and Hori K. (1989) *Biochem. Biophys. Acta.* **1007**, 91-98.

Benfield P.A., Forcina B.G., Gibbons I., and Perham B.L. (1979) *Biochem. J.* **183**, 429-444.

Bewley G.C. and Cook J.L. (1990) *Isozymes: Structure, Function, and Use in Biology and Medicine*, ed. Ogita Z. and Markert C.L. (Wiley-Liss, Inc., NY), pp.341-374. in

Isozymes; Function and use in biuology and medicine:Molecular structure, developmental regulation, and evolution of the gene encoding glycerol-3-phosphate dehydrogenase isozymes in Drosophila melanogaster, ed

Birnstiel M.L., Busslinger M. and Strub K. (1985) *Cell* **41**, 349-359.

Brenner-Holzach O. and Leuthardt F. (1967) *Helvetica Chimica Acta* **50**, 1366-1372.

Brenner-Holzach O. and Leuthardt F. (1969) *Helvetica Chimica Acta* **52**, 1273-1281.

Brenner-Holzach O. and Leuthardt F. (1972) *Eur. J. Biochem.* **31**, 423-426.

Brenner-Holzach O. (1979a) *Arch. Biochem. Biophys.* **194**, 321-327.

Brenner-Holzach O. (1979b) *Arch. Biochem. Biophys.* **194**, 328-335.

Brenner-Holzach O. and Zumsteg C. (1982) *Arch. Biochem. Biophys.* **214**, 89-101.

Brenner-Holzach O. and Smit J.D.G. (1982) *J. Biol. Chem.* **257**, 11747-11749.

Burgess D.G. and Penhoet E.E. (1985) *J. Biol. Chem.* **260**, 4604-4614.

Cook J.L., Bewley G.C., and Shaffer J.B. (1988) *J. Biol. Chem.* **263**, 10858-10864.

Drechsler E.R., Boyer P.D., Kowalsky A.G. (1959) *J. Biol. Chem.* **234**, 2627-2634.

Flechner A., Gross W., Martin W.F. and Schnarrenberger C. (1999) *FEBS Lett.* **447** (2-3): 200-2.

Forcina B.G. and Perham R.N. (1971) *FEBS. Lett.* **18**, 59-63.

Gamblin S.J., Cooper B., Miller J.R., Davis G.J., Littlechild J.A., and Watson H.C. (1990) *FEBS. Lett.* **262**, 282-286.

Gamblin S.J., Davis G.J., Grimes J.M., Jackson R.M., Littlechild J.A., and Watson H.C. (1991) *J. Mol. Biol.* **219**, 573-576.

Gautron S., Maire P., Hakim V., and Kahn A. (1991) *Nuc. Acids Res.* **19**, 767-774.

Gibbons I., Perham R.N., and Anderson P.J. (1972) *Nature New Biol.* **238**, 173-174.

Gracy R.W., Lacko A.G., Brox L.W., Adelman R.C., and Horecker B.L. (1970) *Arch. Biochem. Biophys.* **136**, 480-490.

Gregori C., Ginot F., Decaux J.-F., Weber A., Berbar T., Kahn A., and Pichard A.-L. (1991) *Biochem. Biophys. Res. Commun.* **176**, 722-729.

Gross W., Lenze D., Nowitzki U., Weiske J. and Schnarrenberger C. (1999) *Gene* **230**(1):7-14.

Guha A., Lai C.Y., and Horecker B.L. (1971) *Arch. Biochem. Biophys.* **147**, 692-706.

Henry I., Galano P., Besmond C., Well D., Mattei M.G., Turleau C., Boue J., Kahn A., and Junien C. (1985) *Ann. Hum. Genet.* **49**, 173-180.

Hester G., Brenner-Holzach, Rossi F.A., Struck-Donatz M., Winterhalter K.H., Smit J.D.G., and Piontek Klaus. (1991) *FEBS. Lett.* **292**, 237-242.

Hidaka S., Kadowaki K., Tsutsumi K., and Ishikawa K. (1990) *Nuc. Acids Res.* **18**, 3991-3991.

Horecker B.L., Tsolas O. and Lai C.Y. (1972) in *Enzymes*, ed. Boyer P.D. (Academic Press, NY), Vol. 7, pp.213-258.

Hori K., Mukai T., Joh K., Arai Y., Sakakibara M., and Yatsuki H. (1987) in *Isozymes: Structure and Expression of Human Aldolase Isozyme Genes: Multiple mRNA Species of Aldolase A Produced from a Single Gene*, ed. Scandalios J.G. and Whitt G.S. (Alan R Liss, NY), pp.153-175.

Ikehara Y., Endo H., and Okada Y. (1970) *Arch. Biochem. Biophys.* **136**, 491-497.

Joh K., Mukai T., Yatsuki H., and Hori K. (1985) *Gene (Amst.)* **39**, 17-24.

Joh K., Arai Y., Mukai T., and Hori K. (1986) *J. Mol. Biol.* **190**, 401-410.

Joh K., Mukai T., Motomura M., Oh-uchida M., Yatsuki H., and Hori K.(1990) in *Isozymes: Structure, Function, and Use in Biology and Medicine*, ed. Ogita Z. and Markert C.L. (Wiley-Liss, Inc., NY), pp.53-73.

Kai T., Sugimoto Y., Koga K., and Hori K. (1991a) in *the 64th annual meeting of the Japanese Biochemical Society: The analysis on the aldolase gene structure for Drosophila*

melanogaster, Tokyo, Japan, Oct.1-5; *Seikagaku* (in Japanese) **63**, 1013.

Kai T., Sugimoto Y., Koga K., and Hori K. (1991b) in *the 14th annual meeting of the Molecular Biology Society of Japan: The gene structure and isozyme expression for Drosophila melanogaster aldolase*, Fukuoka City, Japan, Dec.17-20; Program (in Japanese) **1120**, p148.

Kai T., Sugimoto Y., Kusakabe T., Zhang R., Koga K., Hori K. (1992) *J. Biochem.* **112**, 677-688.

Kawahara K. and Tanford C. (1966) *Biochemistry* **5**, 1578-1584.

Kelly P.M. and Tolan D.R. (1986) *Plant Physiol.* **82**, 1076-1080.

Kim J., Yim J.J., Wang S., and Dorsett D. (1992) *Mol. Cell. Biol.* **12**, 773-783.

Kitajima Y., Takasaki Y., Takahashi I., and Hori K. (1990) *J. Biol. Chem.* **265**, 17493-17498.

Kruger I. and Schnarrenberger C. (1983) *Eur. J. Biochem.* **136**, 101-106.

Kukita A., Yoshida M.C., Fukushige S., Sakakibara M., Joh K., Mukai T.,and Hori K. (1987) *Hum. Genet.* **76**, 20-26.

Kukita A., Mukai T., Miyata T., and Hori K. (1988) *Eur. J. Biochem.* **171**, 471-478.

Kusakabe T. (1990) *Master Thesis: Aldolase Genes in Drosophila melanogaster* in Laboratory of Sericulture, Faculty Agriculture, Kyushu University.

Lai C.Y. and Oshima T. (1971) *Arch. Biochem. Biophys.* **144**, 363-374.

Lai C.Y. and Chen C. (1971) *Arch. Biochem. Biophys.* **144**, 467-475.

Lai C.Y. and Horecker B.L. (1972) in *Essays in Biochemistry*, ed. Campbell P.N. and Dickens F. (Academic Press, NY), pp.149-178.

Lai C.Y. (1975) *Arch. Biochem. Biophys.* **166**, 358-368.

Lebherz H.G. and Rutter W.J. (1969) *Biochem.* **8**, 109-121.

Lebherz H.G. (1975) in *Isozymes*, ed. Markert C.L. (Academic Press, NY), pp.253-279.

Lebherz H.G., Bates O.J., and Bradshaw R.A. (1984a) *J. Biol. Chem.* **259**, 1132-1135.

Lebherz H.G., Leadbetter M.M., and Bradshaw R.A. (1984b) *J. Biol. Chem.* **259**, 1011-1017.

Lee Y., Felicioli R., and Horecker B.L. (1975) *Arch. Biochem. Biophys.* **166**, 592-598.

Lipman D.J. and Peaarson W.R. (1985) *Science* **227**, 1435-1441.

Maire P., Gautron S., Hakim V., Gregori C., Mennecier F., and Kahn A. (1987) J. *Mol. Biol.* **197**, 425-438.

Malek A.A., Suter F.X., Frank G., and Brenner-Holzach O. (1985) *Biochem. Biophys. Res. Commun.* **126**, 199-205.

Mareschal J.-C., Schonne E., and Crichton R.R. (1975) *FEBS. Lett.* **54**, 97-99.

Matsushima T., Kawabe S., Shibuya M., and Sugimura T. (1968) *Biochem. Biophys. Res. Commun.* **30**, 565-570.

Mavridis M., Hatada H., Tulinsky A., and Lebioda L. (1982) *J. Mol. Biol.* **162**, 419-444.

Mo Y., Harris B.G., and Gracy R.W. (1973) *Arch. Biochem. Biophys.* **157**, 580-587.

Morse D.E. and Horecker B.L. (1968) *Arch. Biochem. Biophys.* **125**, 942-963.

Motoki K., Kitajima Y., and Hori K. (1993) *J. Biol. Chem.* in press: Structures and functions of isozyme-specific modules on human aldolase A molecule.

Mukai T., Joh K., Arai Y., Yatsuki H., and Hori K. (1986) *J. Biol. Chem.* **261**, 3347-3354.

Mukai T., Yatsuki H., Arai Y., Joh K., Matsuhashi S., and Hori K. (1987) *J. Biochem.* **102**, 1043-1051.

Mukai T., Arai Y., Yatsuki H., Joh K., and Hori K. (1991a) *Eur. J. Biochem.* **195**, 781-787.

Mukai T., Yatsuki H., Masuko S., Arai Y., Joh K., and Hori K. (1991b) *Biochem. Biophys. Res. Commun.* **174**, 1035-1042.

Numazaki M., Tsutsumi K., Tsutsumi R., and Ishikawa K. (1984) *Eur. J. Biochem.* **142**, 165-170.

Ohno S. (1973) *Nature* **244**, 259-262.

Ohno S., Wolf U., and Atkin N.B. (1986) *Hereditas* **59**, 169-187.

Paolella G, Buono P., Mancini P., Izzo P., and Salvatore F. (1986) *Eur. J. Biochem.* **156**, 229-235.

Penhoet E.E., Rajkumar T., and Rutter J.W. (1966) *Proc. Natl. Acad. Sci. U.S.A.* **56**, 1275-1282.

Penhoet E.E., Kockman M., Valentine R., and Rutter W.J. (1967) *Biochemistry* **6**, 2940-2949.

Proudfoot N. (1991) *Cell* **64**, 671-674.

Rottmann W.H., Deselms K.R., Niclas J., Camerato T., Holman P.S., Green C.J., and Tolan D.R. (1987) *Biochimie (Paris)* **69**, 137-145.

Rutter W.J., Richards O.C., and Woodfin B.M. (1961) *J. Biol. Chem.* **236**, 3193-3197.

Rutter W.J., Blostein R.E., Woodfin B.M., and Weber C.S. (1963) *Advan. Enzyme Reg.* **1**, 39-56.

Rutter W.J. (1964) *Fed. Proc.* **23**, 1248-1257.

Sakakibara M., Mukai T., and Hori K. (1985a) *Biochem. Biophys. Res. Commun.* **131**, 413-420.

Sakakibara M., Mukai T., Yatsuki H., and Hori K. (1985b) *Nucleic Acids Res.* **13**, 5055-5069.

Sakakibara M., Takahashi I., Takasaki Y., Mukai T., and Hori K. (1989) *Biochim. Biophys. Acta.* **1007**, 334-342.

Sawyer L., Fothergill-Gilmore L.A., and Freemont P.S. (1988) *Biochem. J.* **249**, 789-793.

Schapira F., Dreyfus J.C., and Schapira G. (1963) *Nature* **200**, 995-996.

Schapira F. (1981) in *Isozymes*, ed. Rattazzi M.C., Scandalios J.G., and Whitt G.S. (Alan R Liss, NY), pp.27-75.

Schnarrenberger C. (1987) *Regulation and Structure of Isozymes of Sugar Phosphate Metabolism in Current Topics in Biological and Medica;l Research: Cellular Localization, Metabolism, and Physiology*, ed. Rattazzi M.C., Scandalios J.G., and Whitt G.S. (Alan R Liss, NY), pp.223-240.

Shaw-Lee R., Lissemore J.L., Sullivan D.T., and Tolan D.R. (1992) *J. Biol. Chem.* **267**, 3959-3967.

Sheets M.D., Ogg S.C., and Wickens M.P. (1990) *Nuc. Acids Res.* **18**, 5799-5805.

Shiokawa K., Kajita E., Hara H., Yatasuki H. and Hori K. (2002) *Cell Research.* **12**:85-96.

Stuart D.I., Levine M., Muirhead H., and Stammers D.K. (1979) *J. Mol. Biol.* **134**, 109-142.

Stribling D. and Perham R.N. (1973) *Biochem. J.* **131**, 838-841.

Sugimoto Y., Kusakabe T., Koga K., Nishida H., and Hori K. (1990) in *the 34th annual meeting of the Agricultural Chemical Society of Japan: The study of aldolase isozyme for Drosophila and Bombyx*, Fukuoka City, Japan, Mar.31-Apr.2; *Nippon Nogeikagaku Kaishi* (in Japanese) **64**, 37.

Sugimoto Y., Kusakabe T., Kai T., Okaura T., Koga K. and Hori K. (1995) *Arch. Biochem Biophys.* **323**(2):361-366.

Sygusch J., Beaudry D., and Allaire M. (1987) *Proc. Natl. Acad. Sci. U.S.A.* **84**, 7846-7850.

Takahashi I., Takasaki Y., and Hori K. (1989) *J. Biochem.* (Tokyo) **105**, 281-286.

Takasaki Y. and Hori K. (1990) *Protein Engineering* **3**, 273-277.

Takasaki Y., Kitajima Y., Takahashi I., Sakakibara M., Mukai T., and Hori K. (1990a) in *Isozymes: Structure, Function, and Use in Biology and Medicine*, ed. Ogita Z. and Markert C.L. (Wiley-Liss, Inc., NY), pp.935-953.

Takasaki Y., Takahashi I., Mukai T., and Hori K. (1990b) *Prog. Clin. Biol. Res.* **344**, 935-953.

Takasaki Y. and Hori K. (1992) *Protein Engineering* **5**, 101-104.

Ting S.-M., Lai C.Y., and Horecker B.L. (1971a) *Arch. Biochem. Biophys.* **144**, 476-484.

Ting S.-M., Sia C.L., Lai C.Y., and Horecker B.L. (1971b) *Arch. Biochem. Biophys.* **144**, 485-490.

Tolan D.R., Amsden A.B., Putney S.D., Urdea M.S., and Penhoet E.E. (1984) *J. Biol. Chem.* **259**, 1127-1131.

Tolan D.R., Niclas J., Bruce B.D., and Lebo R.V. (1985) *Am. J. Hum. Genet.* **41**, 907-924.

Tsutsumi K., Mukai T., Tsutsumi R., Hidaka S., Arai Y., Hori K., and Ishikawa K. (1985) *J.*

Mol. Biol. **181**, 153-160.

Yatsuki H., Outida M., Atsuchi Y., Mukai T., Shiokawa K. and Hori K. (1998) *Biochem Biophys Acta.* **1442**: 199-217.

クラスⅠアルドラーゼ遺伝子の構造および機能進化

甲斐　達男

＜要　旨＞

FBPアルドラーゼ（EC4.1.2.13）は、ほぼ全ての生物に広く分布する解糖系酵素のひとつである。これは六炭糖の2または1リン酸、つまり、FBPまたはF1Pの分解を可逆的に触媒して、ジヒドロキシアセトンリン酸、および、グリセルアルデヒド-3-リン酸あるいはグリセルアルデヒドを生じるものである。本酵素はアイソザイムシステムの理想的なモデルのひとつとして捕らえられており、比較生物学の立場から、アイソザイムの進化論的理解とともに、酵素機能の分子メカニズムを把握するために膨大な数の研究がなされてきている。このような目的のために、アルドラーゼの遺伝子構造およびmRNA発現についてもよく研究されてきている。本稿ではアルドラーゼについてこれまで蓄積されてきた知見、つまり、一般的な生物のアルドラーゼの分類、特に遺伝子構造と発現に関して脊椎動物アルドラーゼの特徴についてとりまとめ、最後に黄色ショウジョウバエのアルドラーゼに関して脊椎動物と昆虫を比較することにより、その遺伝子進化について言及した。

キーワード：アルドラーゼ、クラスⅠ、遺伝子、進化