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Title	Developmental Changes of Lactate Dehydrogenase (LDH) Isozymes in the Hybrid between Masu Salmon (<i>Oncorhynchus masou</i>) and Pink Salmon (<i>O. gorbuscha</i>)
Author(s)	ARAI, Katsutoshi; 荒井, 克俊; YAMAZAKI, Fumio et al.
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**Developmental Changes of Lactate Dehydrogenase (LDH) Isozymes
in the Hybrid between Masu Salmon (*Oncorhynchus masou*)
and Pink Salmon (*O. gorbuscha*)**

Katsutoshi ARAI* and Fumio YAMAZAKI*

Abstract

There was a conspicuous electrophoretic difference in LDH-B isozyme system between masu salmon and pink salmon. We proved by the use of this distinction as a genetic marker that only a maternal isozyme could be detected during the early embryonic stages and isozymes containing paternal B¹ subunit were expressed at the early tail bud stage in the development of masu ♀ × pink ♂ hybrid salmon. In the same stage, pink salmon demonstrated a change from the binomially distributed pattern to the B₂² homotetramer dominant one. These facts suggest that LDH expression in earlier stages of salmon development depends on the maternal cytoplasm and the beginning of genomic function on the LDH isozyme may be in the tail bud stage.

Isozymic analyses during embryogenesis have been carried out in many species of animals including several teleost fishes.¹⁾²⁾ These investigations demonstrated that there is a time schedule of gene activation responsible for isozymic expression and this expression during development is associated with specific embryonic differentiation. Attempts to estimate the time of maternal or paternal gene activation have been made in inter and intraspecific hybrids utilizing different isozymic patterns as genetic markers. Wright and Moyer³⁾⁴⁾ reported in frog hybrid that only maternal lactate dehydrogenase (LDH) was detected from the beginning of embryogenesis and paternal LDH appeared at the later stages of development. Similar phenomena were also observed in teleosts not only in LDH but also in other isozymic systems.⁵⁾ The present study was carried out to analyze changes of LDH isozyme patterns during ontogeny of pink salmon (*Oncorhynchus gorbuscha*), masu salmon (*O. masou*) and their hybrids in order to determine the time of LDH genomic expression.

Materials and Methods

Five females, 24.5 cm to 40.1 cm in fork-length, and one male, 26.5 cm in fork-length, masu salmon (*Oncorhynchus masou*) were obtained from the Mori branch of the Hokkaido Fish Hatchery in Mori, 30 km north of Hakodate. Two females and one male pink salmon (*O. gorbuscha*), 51.5 cm-60.0 cm in fork-length, were provided by the Oshima branch of the Hokkaido Salmon Hatchery in Yakumo, 70 km north of Hakodate. All the mature pink salmon used were caught from Yurapp river, Yakumo.

* *Laboratory of Embryology and Genetics, Faculty of Fisheries, Hokkaido University*
(北海道大学水産学部発生学遺伝学講座)

Masu salmon were transported by car from Mori to Yakumo for artificial insemination. Hybridization between these two species was carried out by the dry method on September, 29, 1977. The resultant combinations were masu ♀ × pink salmon ♂ and pink ♀ × masu salmon ♂. A small part of the eggs obtained from parental species of masu and pink salmon were also similarly inseminated as controls at the same time and by the same procedure. After insemination, eyes, hearts, livers, kidneys and muscles were taken from the fish used for mating and frozen at -20°C until electrophoretic analysis.

Fertilized eggs were transported to the laboratory of the Faculty of Fisheries, Hokkaido University, Hakodate, and reared at an average water temperature of $10 \pm 1^{\circ}\text{C}$ during the experimental period from September 30 to December 3, 1977. Hatching activity occurred on the 37th day for masu salmon, the 45th day for both hybrids and the 57th day for pink salmon. Final survival rates at 60 days after fertilization in each combination were as follows: Pink salmon, 84%, pink ♀ × masu ♂, 77%, masu ♀ × pink ♂ 15%, masu salmon, 37%. Five to twenty five egg samples were taken from both the controls and the hybrid combinations at 3 day intervals from the 4th to the 30th day after fertilization, then once every 6 days until they were 60 days old. The sampled eggs were dissected and embryonic parts with a little adjacent yolk were collected and frozen at -20°C . In addition, five-egg samples were also taken and fixed with Bouin's solution for morphological observations to determine the developmental stage when the isozymic pattern changes.

The "cell-lysate"⁶⁾ of collected embryos and adult tissues was absorbed by a small piece of Toyo filter paper No. 51 and used for LDH analyses by electrophoresis. Horizontal starch gel electrophoresis was carried out at 4°C for 3 hours at 4 mA/cm in a 12% gel of Amylan starch (Joko Industry Co.). Continuous buffer system (pH. 8.5) was adopted. Gel buffer consisted of 0.03 M borate and 0.005 M sodium hydroxide. Electrode buffer (bridge solution) contained 0.3M borate and 0.085 M sodium hydroxide. After the electrophoresis, the gel was sliced horizontally in 1 mm thicknesses and incubated at 37°C for 2 hours in a dark place in the staining mixture of LDH containing 40 ml of 0.05M Tris-HCl buffer (pH. 8.7), 3 ml of 0.5 M sodium lactate, 12 mg of nicotinamide adenine dinucleotide, 4 mg of nitro blue tetrazolium and 2 mg of phenazine methosulfate.

Results

LDH isozyme patterns

Isozyme patterns of lactate dehydrogenase (LDH) in adult pink and masu salmon were demonstrated in eyes, hearts, livers, kidneys and muscles (Fig. 1). LDH-A isozyme system was observed only in muscle tissues and separated electrophoretically around the origin in both species. These muscle specific LDH-A isozymes resulted from two different subunits controlled by two distinct genetic loci A_1 and A_2 . There seemed to be interspecific differences between LDH-A isozyme systems of the two species but we could not identify allelic distinction because of obscure electrophoretic resolution.

LDH isozymes observed to be ubiquitously in all tissues of adult pink salmon were LDH-B system containing five tetramic isozymes B_1^1 , $B_1^1B_2^1$, $B_2^1B_2^1$

$B_1^1B_2^2$ and B_4^4 except livers in which only B_4^4 homotetramer was expressed. Five banded LDH-B systems observed in pink salmon appeared in a tissue specific manner. Five banded isozymes had almost the same staining intensity in the eye but those detected in the heart were tissue specific with LDH-B¹ dominant. Such a specificity was in the kidney in which LDH-B² was more dominant than LDH-B¹. On the contrary, in masu salmon only the LDH-B⁴ isozyme was observed and B_4^4 homotetramer and $B_3^1B_1^1$, $B_2^2B_2^2$, $B_1^1B_3^3$ heterotetramer found in pink salmon were not detected in any tissue. There was, however, a difference in the isozyme band between liver and other tissues, namely the isozymic band of the liver was thinner than that of the others.

Most anodally migrated LDH-C₄ isozyme was eye specific and did not vary between pink and masu salmon, but there were some differences between these two species in the heterotetramic isozyme bands migrating between C₄ and B₄⁴ (Fig. 1).

Developmental Changes

As shown in Fig. 2, LDH-B heterotetramers of pink salmon were intensively stained in a binomial pattern in the early embryonic stages. This pattern persisted through the morula, blastula, gastrula and neurula stages until at least 12 days after fertilization, then changed to a different pattern in which anodal B₄⁴ homotetramer became dominant in staining from 14 days after fertilization when the embryos were in the early tail bud stage. The muscle specific isozymes were faintly detected 30–40 days after fertilization. In masu salmon, however, only one isozyme corresponding to the B₄⁴ homotetramer of pink salmon was detected in LDH-B system throughout the developmental process (Fig. 3). Muscle specific isozymes were detectable from 26 days after fertilization.

Changes in the LDH isozyme pattern in the development of hybrids between pink ♀ and masu salmon ♂ were almost the same as those observed in pink salmon (Fig. 4). On the other hand, dramatic change in the isozyme pattern was found in hybrids between masu ♀ and pink salmon ♂ (Fig. 5). The pattern in the early development of the embryos until the 12th day (Fig. 6) through the cleavage to the neurula stage was completely the maternal masu salmon pattern, but additional paternal isozyme including B¹ subunit derived from pink salmon was clearly detectable at 14 days after fertilization when the tail bud was formed and the blastopore was completely closed in the developing embryos (Fig. 7). All isozymes containing LDH-B¹ subunit derived from paternal pink salmon completed until 40 days after fertilization, though B₄⁴ homotetramer was always dominant during the whole developmental period. Isozymes belonging to LDH-A system were detected in 40 day embryos. No eye specific isozyme (LDH-C system) was detected during the period from fertilization to hatching.

Discussion

The present study demonstrated conspicuous differences in phenotypes of LDH-B system between masu and pink salmon. Muscle specific LDH-A system, tissue ubiquitous LDH-B system and eye specific LDH-C system of pink salmon described in the present paper closely resemble those reported in the same species by Utter *et al.*⁷⁾ On the contrary, the LDH phenotype of masu salmon was unique

and demonstrated that the isozymes synthesized with LDH-B¹ subunits were missing and only one band, B₂² homotetramer was found in tissue ubiquitous LDH-B system. These facts suggest either the lack of *Ldh*-B₁ locus or almost the same electrophoretic mobility of the products of *Ldh*-B₁ and B₂ genes in masu salmon. The isozymic patterns of masu salmon presented in this study, however, indicated that there was a slight difference in LDH staining between liver and other tissues. In addition, the assumption of the presence of two genetic loci synthesizing different subunits with similar electric charge in the LDH-B system will easily explain the position of each heterotetrameric band appeared between LDH-C₄ and B₂² bands. These evidences suggest the possibility of the presence of two genetic loci in LDH-B system in masu salmon.

The conspicuous difference in LDH-B system between masu and pink salmon greatly benefits developmental analysis as a useful gene marker to estimate the time of paternal *Ldh*-B₁ gene activation, because the LDH-B¹ subunit originates only from the gene activation of paternal pink salmon when female masu salmon are mated with male pink salmon. The observation of isozymic changes during the embryogenesis of this hybrid of masu ♀ × pink ♂ reveals only the existence of the maternal isozyme during early stages of development and the later appearance of paternal B¹ subunit from 14 days after fertilization when the embryos were in the early tail bud stage. Such persistence of maternal isozyme during early development has been reported in frog,³⁾⁴⁾ bird⁸⁾ and other species of teleost fishes.⁹⁾¹⁰⁾ According to Wright and Subtelny,¹¹⁾ this phenomena was explainable by the presence of the maternal isozymes which would be stable cytoplasmic enzymes synthesized during oogenesis and stored in the eggs. The appearance of LDH-B¹ subunit at the tail bud stage may suggest *Ldh*-B₁ gene activation at this stage.

As it was suspected from the hybrid experiment that the isozyme detected during early stages of embryogenesis would originate from what stored in the maternal cytoplasm, the binomial distribution of LDH bands found in pink salmon may be regarded as due to the maternal isozymes composed of equal amounts of subunits B¹ and B². Further, the change to the dominance of B₂² homotetramer observed at the tail bud stage in pink salmon may also suggest the differential activation of *Ldh*-B₂ gene at this stage. Consequently, these isozymic changes found in this stage were understood to reflect the beginning of genomic function concerning LDH phenotypic expression. It is reasonable to surmise, however, that there is a time lag between gene activation and the appearance of gene products, because of the time involved from the transcription of the gene to the appearance of the protein phenotype.

Detailed analyses of gene activation using isozymes as genetic markers will provide more informative evidences in the developmental biology of fishes. These investigations may be useful not only in basic understanding of normal development but also for analysis of genetic deformity or abnormal development in fish.

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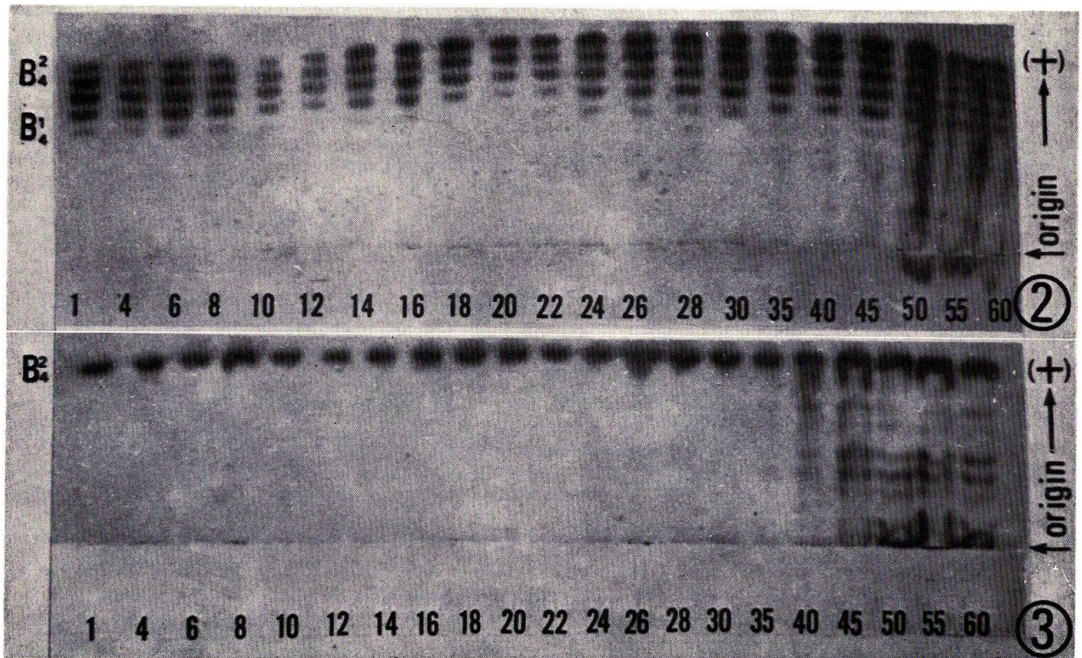
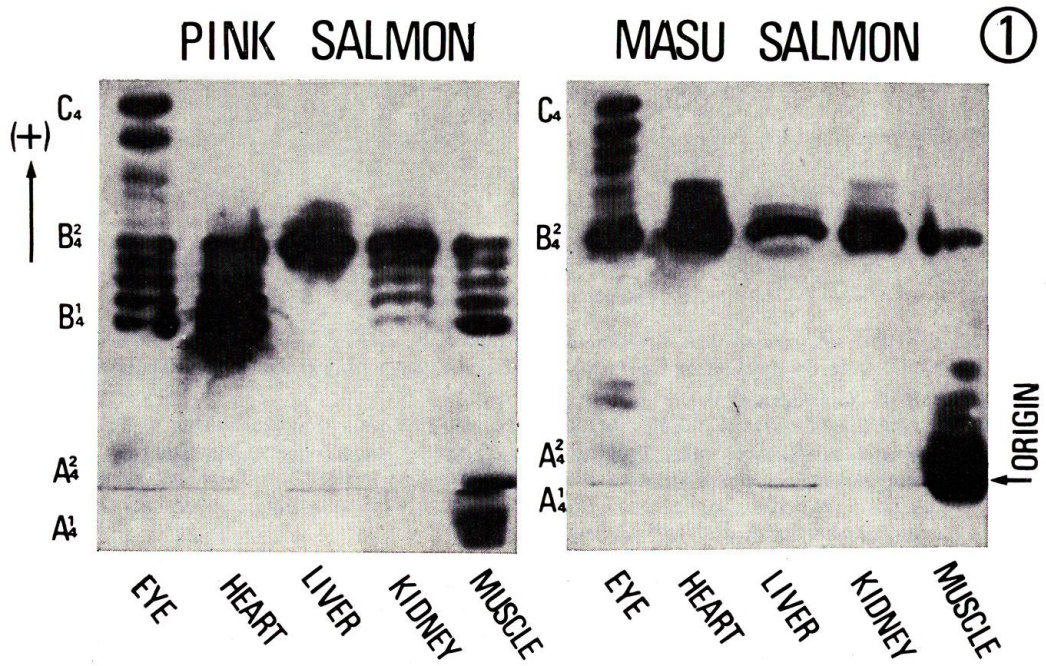
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Explanations of Plate I

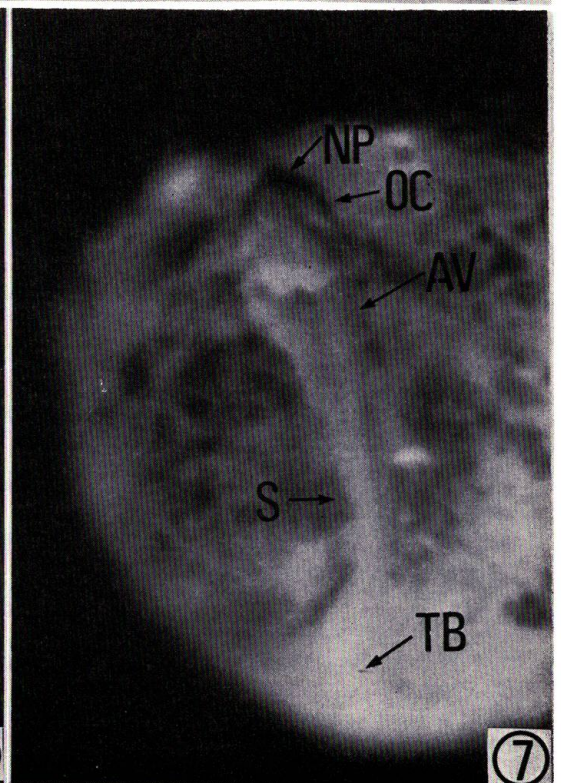
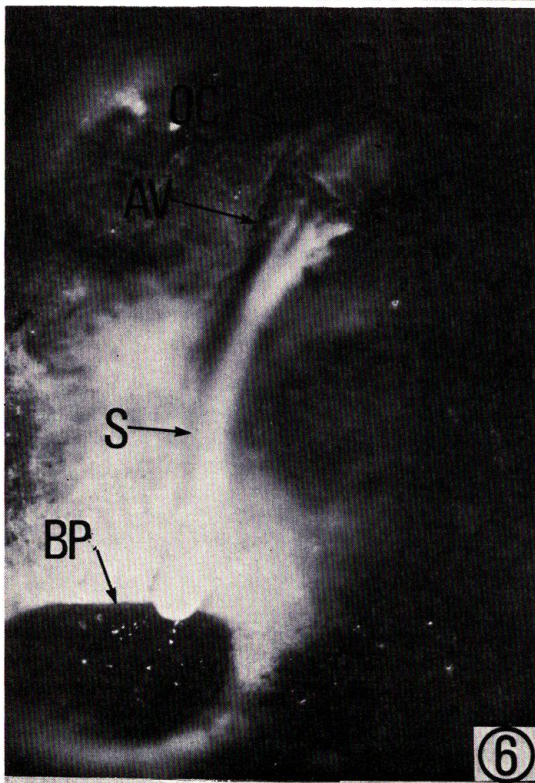
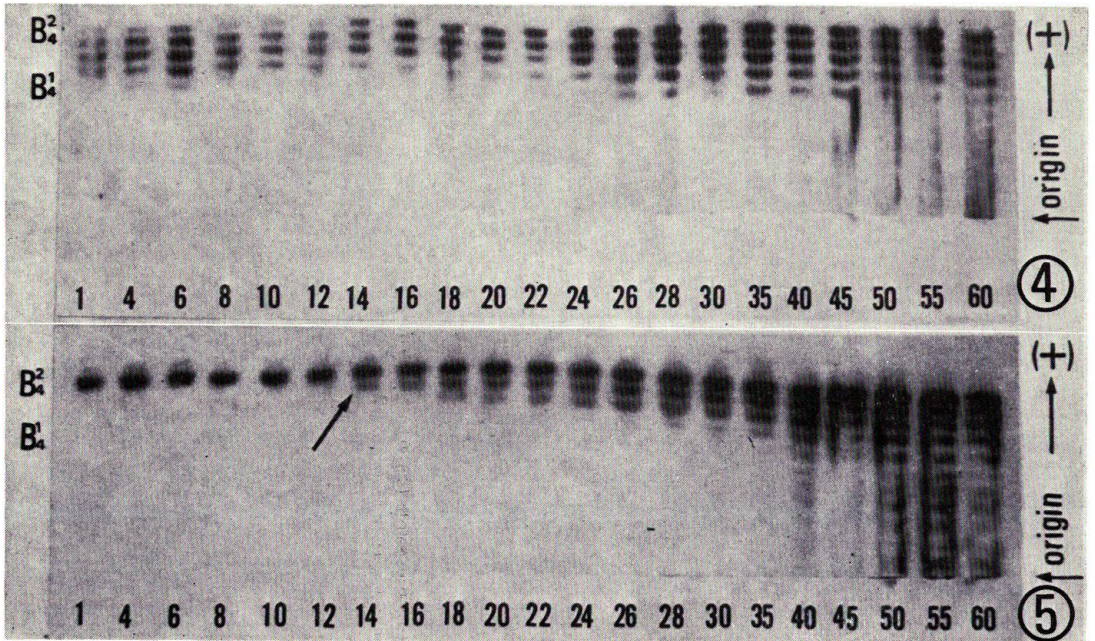
- Fig. 1. Lactate dehydrogenase (LDH) isozyme patterns observed in adult tissues of pink salmon and masu salmon.
- Fig. 2. LDH isozymes in the development of pink salmon during a period from 1 to 60 days after fertilization. Note the dominance of B_4^2 homotetramer from 14 days after fertilization.
- Fig. 3. LDH isozymes in the development of masu salmon during a period from 1 to 60 days after fertilization.



ARAI & YAMAZAKI: LDH isozyme in hybrid salmon

Explanations of Plate II

- Fig. 4. LDH isozymes in the development of pink ♀ × masu ♂ hybrid during a period from 1 to 60 days after fertilization. The 14 day embryo shows the dominance of B_4^2 homotetramer.
- Fig. 5. LDH isozymes in the development of masu ♀ × pink ♂ hybrid during a period from 1 to 60 days after fertilization. Note the persistence of the isozyme of maternal masu salmon type until 12 days after fertilization and new appearance of paternally derived isozymes from 14 days after fertilization. Arrow indicates the first appearance of the paternal isozyme.
- Fig. 6. External appearance of the embryo of masu ♀ × pink ♂ hybrid at 12 days after fertilization. Optic cup (OC); auditory vesicle (AV); somite (S); blastopore (BP).
- Fig. 7. External appearance of the embryo of masu ♀ × pink ♂ hybrid at 14 days after fertilization. Note the enclosure of the blastopore and formation of the tail bud. Nasal placode (NP); optic cup (OC); auditory vesicle (AV); somite (S); tail bud (TB).



ARAI & YAMAZAKI: LDH isozyme in hybrid salmon