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[SHORT COMMUNICATON]

Expression of *DMY* and *DMTR1* in Various Tissues of the Medaka (*Oryzias latipes*)

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ABSTRACT—Two DM-domain genes, *DMY* (sex-determining gene) and *DMRT1*, have been reported to be expressed in the testis of medaka. In this study, a specific RT-PCR assay was used to determine the expression patterns of *DMY* and *DMRT1* in various tissues of medaka during different stages of development. The results show that the transcripts of both *DMY* and *DMRT1* are present not only in testes but also in several other tissues from fry and adults of medaka.

Key words: sex-determining gene, testis, teleost fish, gonadal somatic cell, DM-domain genes

INTRODUCTION

Sex-determining genes (*SRY/Sry*) have been identified in mammals (Sinclair *et al*., 1990; Koopman *et al*., 1991). However, no comparable genes have been found in non-mammalian vertebrates. Using positional cloning, we recently identified the sex-determining gene of medaka (Matsuda *et al*., 2002). This gene encodes a protein that contains a DNA-binding motif called a DM-domain, which was originally described as a DNA-binding motif shared between *doublesex* (*dsx*) in *Drosophila melanogaster* and *mab-3* in *Caenorhabditis elegans* (Raymond *et al*., 1998). We thus named the sex-determining gene of madaka *DMY* (DM-domain gene on the Y-chromosome). Another DM-domain gene, *DMRT1*, has also been reported to be present in the testis of medaka (Brunner *et al*., 2001; Nanda *et al*., 2002). Despite the great diversity of sex-determining mechanisms involved, *DMRT1* is one of well conserved genes related to sexual differentiation from invertebrates to vertebrates irrespective of whether the initiation signal of sex determination comes from genetic or environmental elements (Zarkower, 2001). In this study, we used a specific RT-PCR assay to examine the expression of two DM-domain genes, *DMY* and *DMRT1*, in various tissues of fry and adults as well as embryos of madaka just before and after hatching. We showed that *DMY* and *DMRT1* transcripts were present not only in testes but also in several other tissues of fry and adults.

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MATERIALS AND METHDS

Fish

Medaka, *Oryzias latipes* (orange-red variety) were kept in fresh water at 27°C.

PCR for Genotyping

Medaka fry's tails were incubated in the buffer (500 mM KCl, 100 mM Tris-HCl, pH 8.0, 0.5% NP40, 0.5% Tween20, 0.5% proteinase K) for 2 hr at 50–60°C and were boiled for 10 min. *DMY* and *DMRT1* were amplified from the sample using one primer pair; PG17-5 (CCGGGTGCCCAAGTGCTCCCGCTG) and PG17-6 (GATCGTCCCTCCACAGAGAAGAGA).

Total RNA Extract

Total RNA was isolated from embryos and various tissues from fry and adults of medaka using the RNeasy Mini Kit (QIAGEN).

RT-PCR

With the Onestep RT-PCR Kit (QIAGEN), *DMY* and *DMRT1* were amplified from the total RNA using one primer pair: PG17-25 (CCCACCAGATCCTATACAAGTGAC) and PG17-24 (GTAAACACGGGCCGCAGCCGATGGGC). To distinguish *DMY* and *DMRT1*, *DMY* was digested by *Nhe* I. The PCR conditions and specific primers for *PG04* were described previously (Matsuda *et al*., 2002).

RESULTS AND DISCUSSION

The similarity between *DMY* and *DMRT1* in both amino acid and nucleotide sequences is very high (Matsuda *et al*., 2002). For this reason, the primers were designed to amplify both of *DMY* and *DMRT1*. To distinguish *DMY* and *DMRT1*, *DMY* was digested by *Nhe* I. The present study using RT-PCR combined with enzyme digestion revealed that *DMY* was expressed in XY males throughout the developmental stages, but not in XX females, confirming that *DMY* is a Y-specific gene.

Expression of *DMY* was evident in male embryos just before (Fig. 1A) and after hatching (Fig. 1B). Two separate portions (the head portion which includes brain and the body portion which includes gonads) were used for the analyses of embryos and fries till 20 days after hatching (dah). As shown in Fig. 1B, *DMY* transcripts were already present in the body portion of males collected just after hatching. This is consistent with the results of our earlier RT-PCR and *in situ* hybridization studies which show that *DMY* mRNA is expressed in testicular somatic cells of embryos and fry of male medaka (Matsuda *et al*., 2002). In medaka, gonadal sex differentiation becomes evident in embryos before hatching; the number of germ cells in females begin to increase at stages 37 and 38 (Kobayashi *et al*., unpublished). Thus, *DMY* expression at these periods may be responsible for the suppression of mitosis of germ cells in males.

DMRT1 expression does not occur in either male or female embryos and fry until 20 dah and becomes evident in testis when tested at 30 dah (data not shown). Thus, there appeared to be no overlapping between expression of *DMY* and *DMRT1* during earlier stages of gonadal sex differentiation. Similar findings were reported in medaka by Nanda *et al*. (2002). However, the expression pattern of *DMRT1* in medaka differs from those of other fishes. For example, in tilapia, *Oreochromis niloticus*, *DMRT1* is expressed in XY gonads prior to the formation of testis, suggesting a significant role for *DMRT1* in the earlier stages of gonadal sex differentiation (Kobayashi *et al*., unpublished). The first appearance of *DMRT1* in medaka at 30–40 dah suggests that *DMRT1* may function in spermatogenesis in

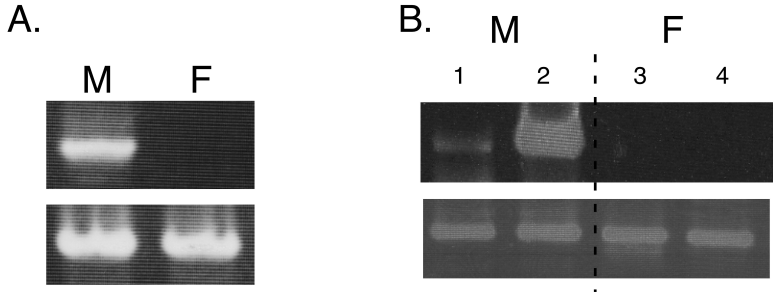

Fig. 1. RT-PCR of *DMY* in medaka embryos. A, *DMY* (top) and *PG04* (bottom, control) transcripts of males (M) and females (F) just before hatching. B, *DMY* (top) and *PG04* (bottom, control) transcripts of males (M) and females (F) just after hatching. Embryos just after hatching were separated into the head (B1, 3) and body (B2, 4) portions.

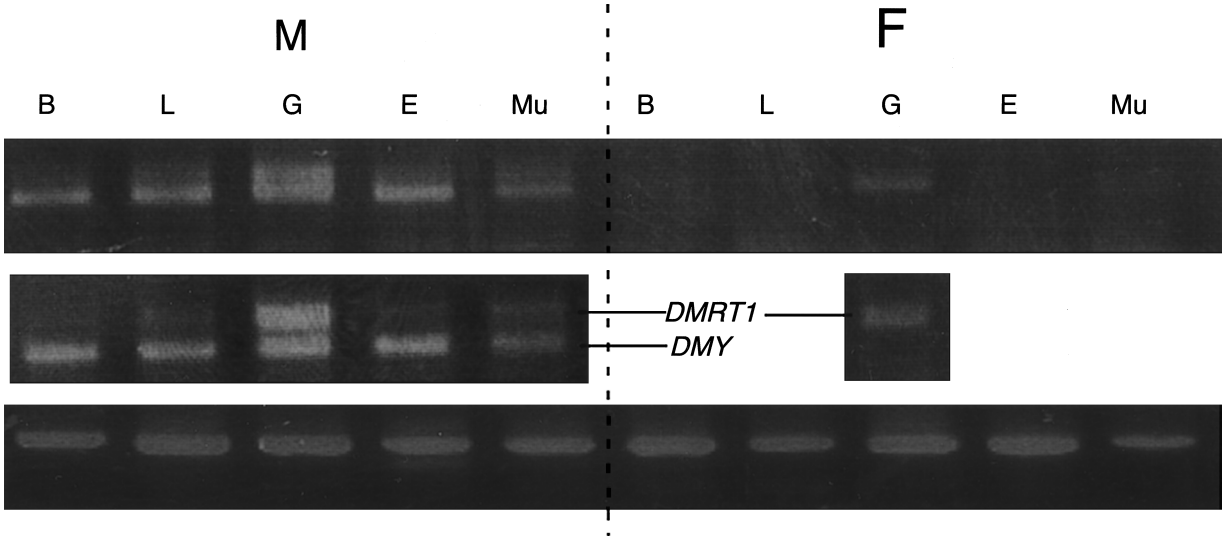

Fig. 2. RT-PCR of *DMY* and *DMRT1* in various tissues of sexually maturing medaka. Samples from males (M) and females (F). Top: Before enzyme digestion. Middle: After enzyme digestion. Bottom: *PG04* control. B, brain; L, liver; G, gonad; E, eyes; Mu, muscle.

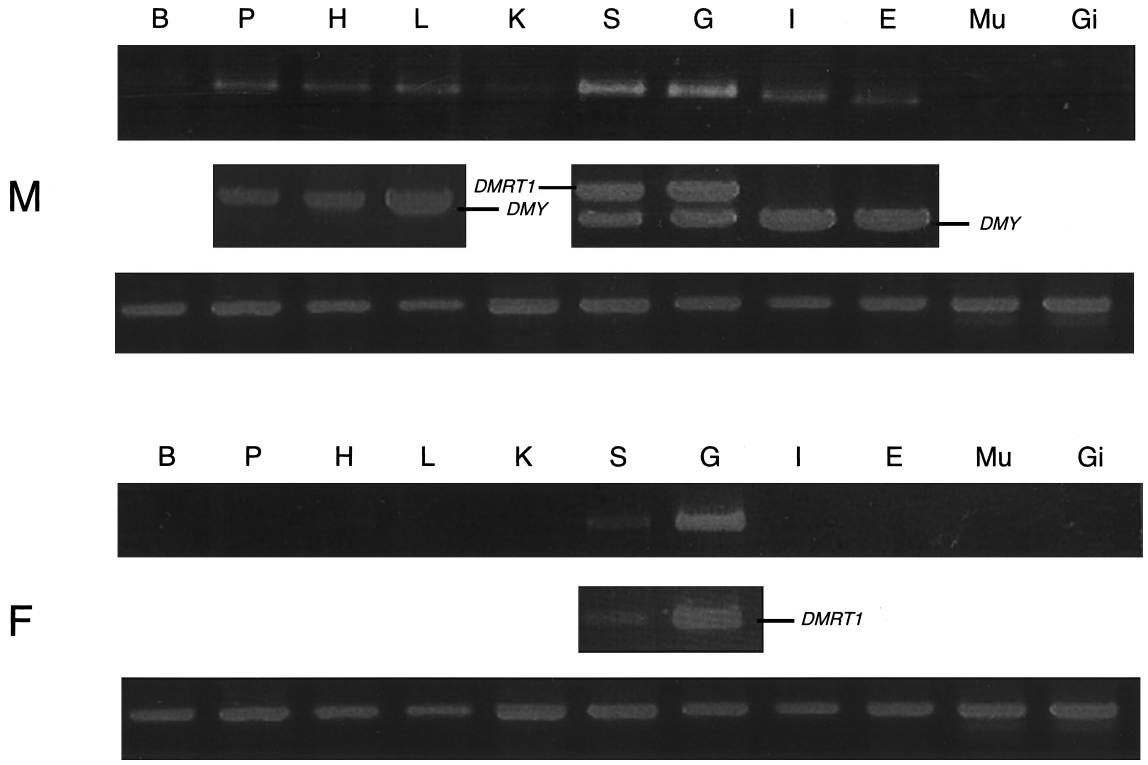

Fig. 3. RT-PCR of *DMY* and *DMRT1* in various tissues of sexually mature medaka. Samples from males (M) and females (F). Top: Before enzyme digestion. Middle: After enzyme digestion. Bottom: *PG04* control. B, brain; P, pituitary; H, heart; L, liver; K, kidney; S, spleen; G, gonad; I, intestine; E, eyes; Mu, muscle; Gi, gill.

medaka.

Under the conditions used in this study, both male and female medaka become sexually mature at about 80-100 dah. In sexually maturing fish sampled from 40 to 75 dah, *DMY* expression was observed not only in testis but also in several other tissues (brain, liver, eye and muscle) of XY males, with the highest expression in testis (Fig. 2). Interestingly, *DMRT1* is also expressed in various male tissues with the highest expression in testis and also in ovary (Fig. 2). The expression patterns of *DMY* in sexually mature fish were similar to those of sexually maturing males, except for a high expression in spleen as well. In the case of *DMRT1*, high expression was evident in testis, ovary and spleen (Fig. 3).

It is of interest that although the sex-determining gene, *DMY*, is expressed only in males, expression does not restrict to testis. Indeed, its expression occurs in many other tissues including brain, liver, eyes, muscle, pituitary, heart, spleen, and intestine, as well as the head portion of embryos and fry that includes the brain. This expression pattern of *DMY* is similar to that of *SRY* in humans, in which *SRY* transcripts are not confined to the presumptive and mature gonadal tissues in the embryo and the adult but are more widely expressed (Clepet *et al*., 1993). In contrast, in the mouse embryo, *Sry* transcripts appear primarily in the genital ridge at a stage corresponding to the onset of testis formation (Koopman *et al*., 1990). The detection of *DMY* transcripts in medaka and *SRY* transcripts in humans, even at low levels, could suggest roles for these sex-determining genes outside testis and genital ridges.

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