Oryzias curvinotus Has DMY, a Gene That Is Required for Male Development in the Medaka, O. latipes

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Source: Zoological Science, 20(2) : 159-161

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.20.159

ZOOLOGICAL SCIENCE **20**: 159–161 (2003)

[SHORT COMMUNICATION]

Oryzias curvinotus Has *DMY*, a Gene That Is Required for Male Development in the Medaka, *O. latipes*

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ABSTRACT—*DMY* is a Y-specific DM-domain gene required for male development and appears to be the sex-determining gene in the teleost fish medaka, *Oryzias latipes*. Although the genomic region containing *DMY* appears to have originated through duplication of the *DMRT1* region, it is unknown when the duplication occurred. Here we show that *O. curvinotus* also has the *DMY* gene on the Y chromosome, which is homologous to the Y chromosome of medaka, and that *DMY* is expressed in XY embryos. A phylogenetic tree based on the amino acid sequence including the DM-domain shows that *DMY* was derived from *DMRT1* immediately before speciation of *O. latipes* and *O. curvinotus*.

Key words: medaka, *DMY, DMRT1*, sex determination

INTRODUCTION

Although the sex-determining gene *Sry* on the Y chromosome has been identified as the testis-determining gene in mammals, no equivalent gene has been found in non-mammalian vertebrates. Recently, we found a Y-linked gene with properties consistent with that of a sex-determining gene in the medaka (*Oryzias latipes*). This gene, named *DMY* (DM-domain gene on the Y chromosome), encoded 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). DM-domain containing genes have also been found in many vertebrate species. One DM-domain containing gene, *DMRT1* (DM-related transcription factor 1) appears to be involved in a sex-determining cascade (Raymond *et al*., 1999; Smith *et al*., 1999; De Grandi *et al*., 2000; Guan *et al*., 2000; Kettlewell *et al*., 2000; Marchand *et al*., 2000; Moniot *et al*., 2000).

In medaka, the nucleotide sequences of *DMY* and *DMRT1* have a similarity of 83% (based on cDNA sequences) and the genomic region containing *DMY* appears to have originated through duplication of the *DMRT1* region (Brunner *et al*., 2001; Nanda *et al*., 2002). We report here phylogenetic relationships between *O. curvinotus DMY* and *DMRT1*, and discuss the time *DMY* acquired its sex-determining function.

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MATERIALS AND METHODS

Linkage analysis

O. curvinotus was maintained by mass mating at Niigata University.

For genotyping, genomic DNA was extracted from a part of the caudal fin as described previously (Matsuda *et al*., 1997).

Genomic PCR was performed using the primers for *O. latipes DMRT1* and *DMY*: PG17.25, CCCACCAGATCCTATACAAGTGAC; PG17.48, GGCTGGTAGAAGTTGTAGTAGGAGGTTT. PCR conditions were 5 min 95°C, followed by 30 cycles of 20 s at 96°C, 30 s at 55°C, 60 s at 72°C, followed by 5 min at 72°C. The primers for *Casp6* were described previously (Kondo *et al*., 2001).

Expression analysis

Genomic DNA and total RNA were extracted from each hatched embryo as described (Matsuda *et al*., 2002). Genomic PCR was performed using primers for *DMRT1* and *DMY* as described above. RT-PCR was performed using a OneStep RT-PCR kit (Qiagen). PCR conditions were 30 min at 55°C; 15 min at 95°C; 20 s at 96°C, 30 s at 55°C, 60 s at 72°C for 30 cycles; and 5 min at 72°C. Approximately 100 ng of total RNA was used as template. For RT-PCR of *DMY*, the same primer set of genomic PCR was used. The PCR conditions and specific primers for *PG04* were described previously (Matsuda *et al*., 2002). RT-PCR products were confirmed by direct sequencing.

Phylogenetic analysis

A phylogenetic tree was constructed using MEGA2 software (Kumar *et al.*, 2001) in which distances were made to be proportional to the number of different amino acids. Human DMRT2 was used as an outgroup.

RESULTS AND DISCUSSION

Because the nucleotide sequence of *DMY* is similar to that of *DMRT1*, many PCR primers can be used on both genes. We found that a PCR primer pair designed for *DMY* and *DMRT1* of medaka could amplify *DMY* and *DMRT1* of *O. curvinotus* (Fig. 1). To determine whether *DMY* is on the Y chromosome of *O. curvinotus*, we first checked the *O. curvinotus* genome of our stock by using PCR of *DMY* and *Casp6*, respectively. *Casp6* is a DNA marker that has been reported to be sex-linked in *O. latipes* and *O. curvinotus* (Kondo *et al.*, 2001). In our stock, males have *DMY* and are heterogametic in *Casp6*, whereas females do not have *DMY* and are homogametic in *Casp6*. We then mated males and females and obtained 57 offspring (30 males and 27 females). All the male progeny had the paternal genotype, whereas all the female progeny had the maternal genotype. This result indicates that *O. curvinotus* also has an XX-XY sex-determining system and that *DMY* is located on the Y chromosome, which is homologous to the medaka Y chromosome.

Furthermore, we obtained cDNA sequences of the coding region of *DMY* (from embryos) and *DMRT1* (from testis) by the RACE method. A cDNA sequence of *DMY* was found to encode a putative protein of 280 amino acids, whereas a cDNA sequence of *DMRT1* was found to encode a putative protein of 276 amino acids (Fig. 2). A phylogenetic tree based on the amino acid sequences of the DM-domains of these and other DMY and DMRT1 in the database indicated (Fig. 3) that *Oryzias* DMY made a clade with *Oryzias* DMRT1. The monophyly of *Oryzias* DMY/DMRT1 was sup-

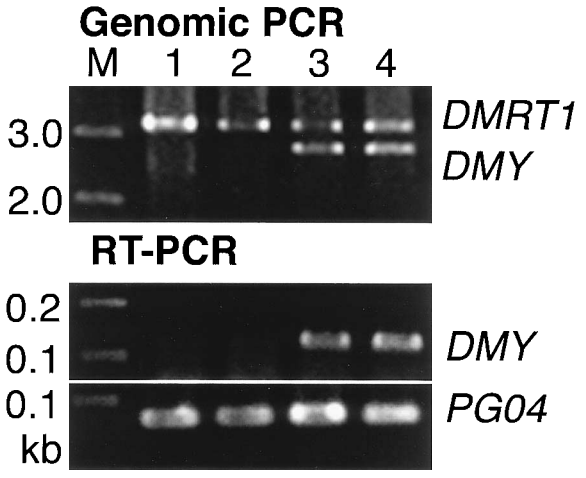

Fig. 1. Genomic and RT-PCR analyses of *DMRT1* and *DMY* of embryos just after hatching. Some of results are shown. Lanes 1–4 show each embryo. Genomic PCR products were electrophoresed in a 1% agarose gel with a 1-kb DNA ladder, whereas RT-PCR products were electrophoresed in a 2% agarose gel with a 100-bp DNA ladder.

```
                    10         20         30         40
DMRT1(Ocu)MSKEKQCRPVPEGPVPGPQRSPRMPKCSRCRNHGLVSPLK
DMRT1(Ola).....G.......A.....................F.....
DMY (Ocu) ..EK...Q......A..R.....K...F........T...
DMY (Ola) .........G.-----------.V.........S.KT...
          50         60         70         80         90
GHKRFCRWKDCACAKCRLIAERQRVMAAQVALRRQQAQEEELGICSPEA-
.......R....K...G.............................S
.....P.........K..VV.....................-.....S
.........H.L..K..VD.....................-.....S
        100        110        120        130        140
---EVVVKNEAGADCLFSMEGRSGAPAAPPNPIPLSAAGSCPASSSSPSA
SGP..T....T.............T.GV.....--.........S.....
SGP....Q....S...........T..V...H---------S..LC....
SGP.............V.....T..P....N...V...YS.....-...
        150        160        170        180        190
AARVYGEEASDLLLETSYYNFYQPSRYSSYYGNLYNYQQYQQMPPSDGRL
.....................................--.....-.....
T....S.....A...........S.....................-.....
.....S.....QP.....................................
        200        210        220        230        240
SGHSMPSQYRMHSFYPGTAYLPQGLGSPVPPYFSLEDND---A-FPPSSL
.....................................GAAAS.S....
.........C......K.....................GAAAS.CTR..
.....................................GAAAS.F....
          250        260        270        280
TSTHDSTLTGRSISSPVNVGVKAEFESGGQPPVFPADSMSSETK*
.........C.....L...............S..........S..
..P.......H....L......V........S...D.........
.........Y.....L..D.............SSRPTP*
```

Fig. 2. Alignment of DMY and DMRT1 proteins in *O. latipes* (Ola) and *O. curvinotus* (Ocu). Period indicates sequence identity; dash indicates a gap; asterisk indicates a stop codon. The DM-domain occurs at positions 23-76. A single base pair deletion at position 272 of Ola DMY has caused a frame-shift mutation. The DNA Data Bank of Japan (DDBJ) accession numbers of the *O. curvinotus* DMY and DMRT1 cDNA sequences are AB091695 and AB091696.

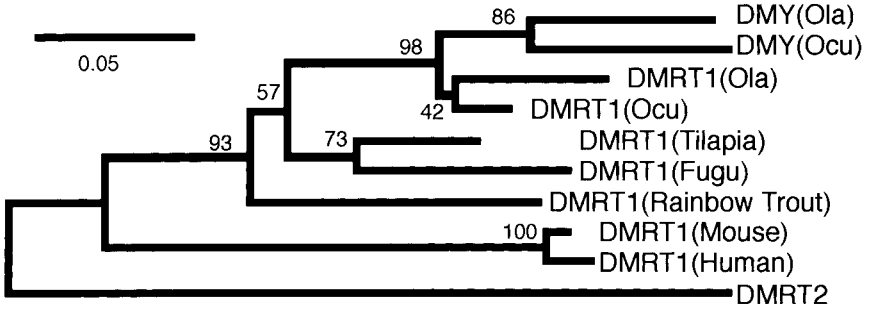

Fig. 3. A neighbor-joining tree based on the amino acid sequences from positions 21 to 109 in Fig. 2. The GenBank accession numbers of the sequences used in the tree were as follows: AF203489 (tilapia DMRT1), AJ295039 (fugu DMRT1), AF209095 (rainbow trout DMRT1), AF202778 (mouse DMRT1), AF130728 (human DMRT1) and AF284223 (human DMRT2). The scores above each branch represent bootstrap values of 1000 times.

ported by a 98% bootstrap value. The clade was divided into two lineages; one consisted of the DMYs of *O. latipes* and *O. curvinotus*, and the other consisted of the DMRT1s of these two species. The bootstrap value of the former clade was high (86%), while that of the latter clade was low (42%). These results suggested that *DMY* was derived from *DMRT1* immediately before speciation of *O. latipes* and *O. curvinotus*.

Oryzias curvinotus has the same sex-determining mechanism as medaka and has *DMY* on the Y chromosome, which suggests that *DMY* also has a role in sex determination of *O. curvinotus*. The branch length of *DMY* is longer than that of *DMRT1* in the phylogenetic tree (Fig. 3). This means that *DMY* has more mutations than *DMRT1* and suggests that *DMY* accumulated these mutations after it acquired its sex-determining function. In other words, the rate of evolution of *DMY*, which is a Y-linked sex-determining gene, is higher than that of *DMRT1*, which is the origin of the Y-linked sex-determining gene. The rate of evolution of *Sry*, which is the sex-determining gene on the Y chromosome of mammals, is also higher than that of *Sox* family genes (Bowles *et al*., 2000). These results suggest that a new sex-determining gene generated by a gene duplication event in the sex chromosome tends to evolve fast.

REFERENCES

Bowles J, Schepers G, Koopman P (2000) Phylogeny of the SOX family of developmental transcription factors based on sequence and structural indicators. Dev Biol 227: 239–255

Brunner B, Hornung U, Shan Z, Nanda I, Kondo M, Zend-Ajusch E, Haaf T, Ropers HH, Shima A, Schmid M, Kalscheuer VM, Schartl M (2001) Genomic organization and expression of the doublesex-related gene cluster in vertebrates and detection of putative regulatory regions for *DMRT1*. Genomics 77: 8–17

De Grandi A, Calvari V, Bertini V, Bulfone A, Peverali G, Camerino G, Borsani G, Guioli S (2000) The expression pattern of a mouse doublesex-related gene is consistent with a role in gonadal differentiation. Mech Dev 90: 323–326

Guan G, Kobayashi T, Nagahama Y (2000) Sexually dimorphic expression of two types of DM (Doublesex/Mab-3)-domain genes in a teleost fish, the Tilapia (*Oreochromis niloticus*). Biochem Biophys Res Commun 272: 662–666

Kettlewell JR, Raymond CS, Zarkower D (2000) Temperature-dependent expression of turtle *Dmrt1* prior to sexual differentiation. Genesis 26: 174–178

Kondo M, Nagao E, Mitani H, Shima A (2001) Differences in recombination frequencies during female and male meioses of the sex chromosomes of the medaka, *Oryzias latipes*. Genet Res Camb 78: 23–30

Kumar S, Tamura K, Jakobsen IB, Nei M (2001) MEGA2: molecular evolutionary genetics analysis software. Bioinformatics 17: 1244–1245

Marchand O, Govoroun M, D'Cotta H, McMeel O, Lareyre J, Bernot A, Laudet V, Guiguen Y (2000) *DMRT1* expression during gonadal differentiation and spermatogenesis in the rainbow trout, *Oncorhynchus mykiss*. Biochim Biophys Acta 1493: 180–187

Matsuda M, Kusama T, Oshiro T, Kurihara Y, Hamaguchi S, Sakaizumi M (1997) Isolation of a sex chromosome-specific DNA sequence in the medaka, *Oryzias latipes*. Genes Genet Syst 72: 263–268

Matsuda M, Nagahama Y, Shinomiya A, Sato T, Matsuda C, Kobayashi T, Morrey CE, Shibata N, Asakawa S, Shimizu N, Hori H, Hamaguchi S, Sakaizumi M (2002) *DMY* is a Y-specific DM-domain gene required for male development in the medaka fish. Nature 417: 559–563

Moniot B, Berta P, Scherer G, Sudbeck P, Poulat F (2000) Male specific expression suggests role of *DMRT1* in human sex determination. Mech Dev 91: 323–325

Nanda I, Kondo M, Hornung U, Asakawa S, Winkler C, Shimizu A, Shan Z, Haaf T, Shimizu N, Shima A, Schmid M, Schartl M (2002) A duplicated copy of *DMRT1* in the sex-determining region of the Y chromosome of the medaka, *Oryzias latipes*. Proc Natl Acad Sci USA 99: 11778–11783

Raymond CS, Kettlewell JR, Hirsch B, Bardwell VJ, Zarkower D (1999) Expression of *Dmrt1* in the genital ridge of mouse and chicken embryos suggests a role in vertebrate sexual development. Dev Biol 215: 208–220

Raymond CS, Shamu CE, Shen MM, Seifert KJ, Hirsch B, Hodgkin J, Zarkower D (1998) Evidence for evolutionary conservation of sex-determining genes. Nature 391: 691–695

Smith CA, McClive PJ, Western PS, Reed KJ, Sinclair AH (1999) Conservation of a sex-determining gene. Nature 402: 601–602

(Received October 18, 2002 / Accepted November 4, 2002)