Sex differentiation of the
gonad of fry transplanted into the anterior
chamber of the adult eye in the teleost,
Oryzias latipes

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SUMMARY

In order to examine whether or not sex differentiation in the medaka, Oryzias latipes, is
modified by the physiological level of sex hormones in the adult fish, trunk regions containing
the gonads of newly hatched fry were transplanted unilaterally into the anterior chamber of
the eyes of adult fish.

The grafts could be classified into two types according to the vascularization. One type of
graft developed well; some of them protruded from the eyes of the host fish. In these grafts
the connexion of the blood circulation between the graft and the host was detectable without
exception. The other type of graft consisted of those specimens without vascularization. The
grafts of this type did not grow in size; this condition seemed to be similar to the so-called
in vivo culture in the anterior chamber of the eye in rodents. Most of these grafts, however,
degenerated.

Judging from the histology of the gonad in the graft, a genetic male graft in the eye of a fish
developed into a testis, regardless of the sexuality of the host. This fact was confirmed by two
series of experiments. The gonad of a genetic female fry developed into an ovary if the graft
was transplanted into a female fish. On the other hand, the gonad of a genetic female graft
transplanted into a male fish failed to develop into an ovary, but formed spermatogenetic cells
in a gonad of an abnormal structure.

Therefore, it is highly probable that the reversal of sex differentiation from genetic oogonia
into spermatogenetic cells is accomplished by the physiological level of the sex hormones
in male fish. On the contrary, the sex reversal of genetic males to females is not induced by the
physiological level of female sex hormones.

INTRODUCTION

In fish, artificial inductions of sex reversal by the administration of exogenous
sex steroids have been accomplished in four species, Oryzias latipes (Yamamoto,
1953, 1958), Lebistes reticulatus (Dzwillo, 1962), Tilapia mossambica (Clemens,
1965) and Carassius auratus (Yamamoto & Kajishima, 1968).

In the medaka, Oryzias latipes, Yamamoto (1953, 1958), using a genetically
analysed strain (d-rR) as the material, succeeded in inducing functional sex

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reversal in genetic males under the influence of exogenous oestrogens and in
 genetic females by the oral administration of androgens at the juvenile stages.
 Hishida (1962) indicated the selective accumulation of testosterone-4-14C in the
gonad, regardless of the sex, only in larval stages.

In order to examine whether or not the modification of sex differentiation
in the medaka is induced by the physiological level of endogenous sex hor-
mones in adult fish, the transplantation of the trunk region, containing the gonad
of a newly hatched fry, into the anterior chamber of the eye of an adult fish was
carried out. The present paper will deal with the development and sex dif-
ferentiation of the gonad in the graft.

MATERIALS AND METHODS

Experiments were carried out with the orange-red variety of the medaka,
Oryzias latipes, an oviparous cyprinodont fish. In this species, the morphologi-
cal sex differentiation of germ cells begins to occur at the time of hatching
(Satoh & Egami, 1972).

For the transplantation, parts of bodies of newly hatched fry kept 5 days
without any food were used as the grafts. The trunk region containing the
gonad of the fry (Fig. 1), 5 mm in total body length, was excised in Ringer’s
solution by means of a sharp razor. In the meantime, a small cut had been made
on the cornea of the right eye of the full-grown male or female fish. The small
piece of the graft was promptly transplanted into the anterior chamber of the
right eye of the adult fish with taper tweezers. No special attention was paid to
the orientation of the graft in the eye. Neither anaesthetic nor disinfectant
management was carried out during the course of the operation. After the
operation about 50 host fish were kept in each cistern, each containing about
40 litres of water, and placed outdoors.

The first and second series of experiments were carried out in the fall of 1971
and in the early summer of 1972 respectively. Ten and 30 days after the opera-
tion in the first experiment, and 10, 25 and 50 days in the second experiment,
hosts were fixed in Bouin’s solution in toto. For histological observations the
right eye was cut out, the sex of the host fish was checked, and the eye was sectioned transversally in paraffin at 6 μm and stained with Delafield's haematoxylin and eosin.

**RESULTS**

1. **Differentiation of the gonad during normal development**

   The morphological sex differentiation of germ cells begins to occur at the time of hatching in this fish. In normal development, male fry immediately after hatching contain about 100 germ cells and none of the cells are in the meiotic prophase, while females have about 200 germ cells, a few of them in the meiotic prophase (Satoh & Egami, 1972).

   Five days after hatching when the transplantation was carried out, the gonad of a fry was suspended in the coelom between the pronephric ducts and the gut in the posterior trunk region, and was obviously identified as a presumptive testis or a presumptive ovary by the histological observation. The former contained merely about 100 germ cells, and each germ cell enclosed by a few of somatic cells (Fig. 2). The latter, however, developed into two lobes of long cyst-like structure, which was oval in shape by the transverse sections (Fig. 3). It consisted of about 300 germ cells in the central region and somatic cells forming the ovarian wall. A few somatic cells were also detectable in the central part of the ovary as young follicle cells. About 35 days after hatching, about 13 mm in total length, the male gonad developed into a typical testis with some acini containing many spermatogonia (Fig. 4), while the ovary of female young contained many developing auxocytes, and a lumen was seen between its stroma and wall (Fig. 5). In other words, the histological structure of the gonad was clearly differentiated.

2. **Differentiation of the gonad in the transplanted fish**

   In both the first and second experiments, about two-thirds of the operated fish survived until the end of the experiments. Most females laid eggs as usual. More than 10 days after transplantation, some grafts developed well and protruded from the eyes of the host fish. Such grafts could be observed from the outside (Fig. 8). Besides, the histological observations of the eyes of some hosts proved the presence of the graft.

   The grafts could be classified into two types (types 1 and 2) according to the vascularization. In the grafts of type 1, the blood circulation of the hosts did not enter into the grafts. Such grafts did not grow in size (Fig. 6a, b). The condition seemed to be similar to the so-called in vivo culture in the anterior chamber of the eye in rodents. One graft of this type in a male host contained 65 germ cells at the primordial spermatogonium stage (Fig. 6b). Most of these grafts, however, degenerated.

   On the other hand, the grafts of type 2 developed well and some of them protruded from the eyes of the hosts (Figs. 7–9). Careful observations of the
serial sections demonstrated the connexion between the blood vessels of the
graft and those of the host without exception. Significant data were obtained
from such successful grafts.

The criteria for deciding the gonad of the graft as a presumptive testis or
ovary were based on the following points. (A) The arrangement of germ cells
and somatic cells in the gonad; gonads in which many somatic cells enclosing
a cluster of germ cells formed each acinus were regarded as testes (e.g. Figs. 4,
9b, 12, 14), while those in which many germ cells existed close to each other
and a layer of somatic cells enclosed the mass of germ cells as ovaries
(e.g. Figs. 3, 5, 13). (B) The number of germ cells and the morphology of germ
cells, especially the state of the nuclei, in the gonad; gonads containing
germ cells in the meiotic prophase were regarded as ovaries, because of the
lack of germ cells in meiosis in presumptive tests within a month in normal
development.

A. The first experiment (fall of 1971)

The results are summarized in Table 1.

(I) Ten days after transplantation. Of 35 surviving operated fish, two males
and two females contained grafts in which the gonads were proven by the
histological observations. Two grafts in the eyes of male hosts contained 88
and 89 spermatogonia respectively in the testes; the structure was similar to
that of a normal presumptive testis at comparable stage of the development.

The gonads of two grafts in the adult females maintained the ovarian struc-
ture containing 85 and 20 oocytes respectively (Table 1).

(II) Thirty days after transplantation. Of 59 surviving fish, 32 contained the
grafts, 14 of them being type 2. Of these, two grafts in male hosts and two in
females contained the germ cells.

The gonad of one graft transplanted into the eye of a female host developed

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**Figures 2-5**

Figs. 2, 3. Transverse sections of the gonads of fry 5 days after hatching, during
normal development.

Fig. 2. Presumptive testis, still under an undifferentiated stage, not containing
germ cells in the meiotic prophase. *Te*, Testis; *Gu*, gut; *PD*, pronephric ducts.

Fig. 3. Differentiating ovary containing germ cells in the meiotic prophase.
It is clear that the arrangement of germ cells and somatic cells is quite different
from that in a testis. *Ov*, Ovary.

Figs. 4, 5. Gonads of normal young 35 days after hatching.

Fig. 4. Typical testis consisting of many acini in which somatic cells enclose
a few of germ cells.

Fig. 5. Typical ovary with many growing oocytes. A lumen is seen between the
ovarian stroma and its wall. *OW*, Ovarian wall; *Lu*, lumen in a ovary.
Transplanted gonad of fish fry

into a typical testis, which consisted of many acini containing 476 spermatogonia (Fig. 9a, b). Another graft in a female host was also observed to have 217 spermatogonia in a differentiating testis.

On the other hand, in the grafts in male hosts no gonadal structure was detectable. The germ cells were in a cluster enclosed by connective tissue.

B. The second experiment (early summer of 1972)

(I) Ten days after transplantation. In two grafts in male hosts, the gonads were found. One graft had a typical presumptive testis containing 62 spermatogonia. The gonad of another graft, however, contained 128 germ cells, 11 of them being in the meiotic prophase. But the development of the ovary was relatively poorer than the ovary of normal fry of the comparative stage.

On the other hand, a graft transplanted into the eye of a female fish contained a typical presumptive testis with 77 spermatogonia.

(II) Twenty-five days after transplantation. Among 215 surviving operated fish, 12 males and 31 females contained grafts of type 2, in which germ cells were detectable. Of 12 grafts with germ cells in the eyes of male hosts, 6 contained histologically normal testes (Fig. 12), while 3 grafts had rudimentary gonads with a smaller number of germ cells. The other 3 grafts in adult males, however, developed abnormal gonads which were quite different in structure from the usual testes or ovaries. The gonads, as a whole, were similar to normal ovaries in their histological appearance. However, two of those gonads contained a few cell-nests filled with primary spermatocytes at the meiotic prophase, their number being 138 and 147 respectively, as well as many cell-nests with spermatogonia (Figs. 11a, b), whereas the other gonad contained a number of cell-nests with spermatogonia. These cell-nests were always detected on the periphery of the gonads, and the central parts of the gonads were occupied by lumens (Figs. 10, 11a, b).

On the other hand, of 31 grafts with germ cells transplanted into the eyes of female hosts, 6 grafts maintained the ovarian structure, containing many oocytes at various stages of prophase in the first meiotic division (Fig. 13). The gonads of the other 5 grafts developed into typical testes (Fig. 14), while the

Figures 6-8

Fig. 6. A graft of type 1 (see the text) in the eye of a male fish 25 days after transplantation.

(a) Total view of the graft. Gr, Graft; Le, lens of the host's eye; Ir, iris; Re, retina.

(b) Higher magnification of the inset region of the Graft shown in (a). A few spermatogonia are seen (arrow).

Fig. 7. A graft of type 2 in the eye of a female fish 25 days after transplantation. Compare to the graft shown in Fig. 6; a well-developed graft.

Fig. 8. Graft (type 2) protruded from the eye of a host fish (arrow).
Fig. 9. Well-developed graft in the eye of a female fish 30 days after transplantation in the first experiment. (a) Total view of the graft. $Gr$, Graft; $Le$, lens of the host’s fish; $Ir$, iris. (b) Higher magnification of the inset region of the graft shown in (9a). A typical testis consisting of many acini is clear. $Te$, Testis.
Table 1. *Number of the grafts (type 2) with gonad*

<table>
<thead>
<tr>
<th>Days after transplantation</th>
<th>Sex of host</th>
<th>No. of hosts</th>
<th>Gonad of graft</th>
<th>No. of gonads</th>
<th>No. of germ cells</th>
<th>Presumed genetic sex of graft</th>
<th>Sex differentiation of gonad</th>
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<tr>
<td>10</td>
<td>Male</td>
<td>2</td>
<td>Testis</td>
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<tr>
<td></td>
<td>Female</td>
<td>2</td>
<td>Ovary</td>
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<td>30</td>
<td>Male</td>
<td>0</td>
<td>—</td>
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<td>—</td>
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<tr>
<td></td>
<td>Female</td>
<td>2</td>
<td>Testis</td>
<td>2</td>
<td>476, 217</td>
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</table>

**First experiment**

**Second experiment**

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<th>10</th>
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<th>2</th>
<th>Testis</th>
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<th>62</th>
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<th>Male</th>
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<tr>
<td></td>
<td>Female</td>
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<td>Testis</td>
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<td>Male</td>
<td>Male</td>
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<tr>
<td>25</td>
<td>Male</td>
<td>12</td>
<td>Testis</td>
<td>6</td>
<td>96, 64, 97, 102, 63, 43</td>
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<td>Male</td>
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<tr>
<td></td>
<td>Female</td>
<td>3</td>
<td>Rudiment</td>
<td>3</td>
<td>471 + 147, 308 + 138, 298</td>
<td>Female</td>
<td>(Female) → Male</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>31</td>
<td>Abnormal gonad</td>
<td>3</td>
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<tr>
<td></td>
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<td>Male</td>
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<tr>
<td></td>
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<tr>
<td></td>
<td>Ovary</td>
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<td>190 + 109, 197 + 23, 203 + 234, 161 + 82, 52 + 30, 61 + 83</td>
<td>Female</td>
<td>Female</td>
<td></td>
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<tr>
<td></td>
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<td>2</td>
<td>Ovary</td>
<td>2</td>
<td>134 + 30, 33 + 42</td>
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Italic figures show the number of oocytes, bold figures the number of primary spermatocytes.
Transplanted gonad of fish fry

Testes of the other 7 grafts were still younger stages. In the other 13 grafts in female hosts, however, the gonads were rudimentary.

(III) Fifty days after transplantation. Most of the well-developed grafts of type 2 did not continue to grow after about 35 days after transplantation, but began to degenerate. Only 8 of 58 grafts were in a good state at the time of fixation, 2 of these grafts containing the gonads.

One of the two grafts in the eye of a female host developed 30 growing auxocytes in the ovary (Fig. 15). The other graft in a female host also contained 42 auxocytes, although the development of the ovarian structure was relatively poor. The genetic sex of these grafts was regarded as female.

The results are also summarized in Table 1.

Discussion

In the present transplantation experiment, the grafts which obtained the direct blood supply from the hosts could continue to develop and to grow. The fact, confirmed by two series of experiments, that the undifferentiated gonads of genetic males developed into testes even in the eyes of adult females seems to indicate that the testes are formed independently of the physiological blood level of the sex hormones in adult females. On the other hand, three grafts in the eyes of male hosts fixed at 25 days after transplantation in the second experiment developed abnormal gonads. Judging from the histology of the gonads, it is highly probable that oocytes of the presumptive ovary reduce and oogonia are accelerated to proliferate, forming the cell-nests of spermatogenetic cells. In other words, the genetic sex of the grafts seems to be female and the reversal of sex differentiation is induced under the influences of sex hormones of the adult males. Therefore, it is concluded that the sex reversal in genetic females is induced by the physiological concentration of androgens in adult males, but that in genetic males is not induced by the physiological blood level of oestrogens in adult females. However, some mechanical conditions of the grafts and the anticipated immunological reaction must also be considered in this experiment.

In the medaka, Oryzias latipes, many data on the mechanism involved in sex...

**Figures 10 and 11**

Figs. 10, 11. Abnormal gonads in the grafts transplanted into adult males; 25 days after transplantation in the second experiment.

Fig. 10. Lower magnification of one of the gonads (area enclosed by a broken line) which is quite different from a normal testis or ovary. Some cell-nests (arrows) on the periphery and lumens in the centre are obvious. Lu, Lumen.

Fig. 11. (a) Another gonad of this type at higher magnification. A cell-nest filled with primary spermatocytes is seen (arrow). CN, Cell-nest. (b) Another section of the same gonad. On the periphery cell-nests are located, while lumens are seen in the central part of the gonad.
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differentiation have been accumulated thanks to Yamamoto and his co-workers. In 1953 Yamamoto successfully induced a functional sex reversal of genetic males by the oral administration of oestrone or stilbestrol. The reversal of the sex differentiation of genetic females by methyltestosterone was also done by Yamamoto (1958). Many kinds of sex-hormone steroids have been shown to be sex inducers in further oral administration experiments (cf. Yamamoto, 1969). Furthermore, Hishida & Kawamoto (1970) showed that 11-ketotestosterone, which appears to be a natural androgen produced in teleostean testis, was most potent as a male-inducer (andro-inducer) among the many androgenic steroids. On the other hand, progesterone and some corticoids gave only negative results in reversing sex differentiation (Yamamoto, 1962). Hishida (1962) indicated the selective accumulation of testosterone-4-14C in the gonad, regardless of sex, in larvae of this fish. Estrone-16-14C and diethylstilbestrol-(monoethyl-l-14C), when injected into the egg, were also concentrated into the gonad during development (Hishida, 1964). In addition, on the basis of the recovery counts of the estrone-16-14C and the diethylstilbestrol-(monoethyl-l-14C) of the larvae, 1.8 x 10^-2 μg estrone and 1.1 x 10^-2 μg diethylstilbestrol respectively were calculated as the oral doses of oestrogens required for a 100% induction of sex reversal in genetic males (Hishida, 1965). Onitake (1972) reported that the gonadogenesis of a genetic male under the influence of estrone was the same as that of a genetic female, judging from the morphological process of the sex reversal. From these experimental facts, they reached the conclusion that the sex hormones were natural sex inducers, androgens as andro-inducers and estrogens as gyno-inducers, in this species (Yamamoto, 1969).

In the series of experiments carried out by Yamamoto and his co-investigators, no direct evidence of the role of endogenous hormones produced by the tissue of Oryzias in sex differentiation was presented. The transplanted gonads in the present experiment developed under the hormonal influences of adult fish of the same species. The results show that, although the differentiation of the presumptive ovary is influenced by the male host, the genetic male gonad differentiates into the testis regardless of the host’s sexuality. The possibility that androgens may act as andro-inducers under natural sex differentiation is suggested.

**Figures 12–15**

Fig. 12. Testis differentiated in the graft in the eye of a male host; 25 days after transplantation. *Te*, Testis.

Fig. 13. Ovary of the graft in the eye of a female host; 25 days after transplantation. *Ov*, Ovary.

Fig. 14. Differentiated testis of the graft in the eye of a female host; 25 days after transplantation.

Fig. 15. Ovary with many growing oocytes of the graft in the eye of a female host; 50 days after transplantation.
It has been found that, in the normal embryonic development in this fish, morphological sex differentiation of germ cells occurs at the time of hatching, but no sexual differences in the somatic elements of the gonad are observable at that time (Satoh & Egami, 1972). From these facts, together with the present results, the possibility still remains that some intracellular mechanisms are also involved in the sex differentiation of germ cells and the gonad in the natural course of sex differentiation.

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REFERENCES


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