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# Developmental Process of Genital Ducts in the Medaka, *Oryzias latipes*

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**ABSTRACT**—The morphological development of genital ducts both intra-gonadal (ovarian cavity and efferent duct) and extra-gonadal (oviduct and sperm duct) was investigated in a model teleost, medaka *Oryzias latipes*. The results showed that the extra-gonadal genital ducts contained two structural units, the anterior and posterior parts, in both sexes. Of special interest is a newly discovered process for the development of a posterior part of the oviduct. The anterior part of oviduct extended continuously from the ovarian cavity at the posterior end of the ovary. Then the posterior part of oviduct, which termed genital pore lip (GPL) in this study, was formed. This part results from invagination and cavitation of the cortex of urinogenital papillae (UGP) and forms the wall of the oviduct opening. We also suggest that the ventral region of urethra mesenchyme has an important role in extra-genital ducts formation.

**Key words:** ovarian cavity, oviduct, genital pore lip (GPL), efferent duct, sperm duct

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## INTRODUCTION

The reproductive system is divided into two functional units, gonads and genital ducts. The gonads produce gametes and the genital ducts carry gametes away from gonads.

In elasmobranchs and tetrapods, the genital ducts develop in close relation to the renal duct system (reviewed in van Tienhoven, 1968). In mammals, for example, the paramesonephric or Müllerian duct differentiates into some parts of the female duct (oviduct, uterus, cervix and upper vagina) and the mesonephric or Wolffian duct differentiates into some parts of the male duct (vas deferens, epididymis, seminal vesicle).

In teleost fish, on the other hand, genital ducts are not derived from the renal duct system and their origins are thought to be somatic cells of the gonad and/or the coelomic epithelium (reviewed in van Tienhoven, 1968; Nagahama, 1983). In the female, the ovary generally has an ovarian cavity, the intra-gonadal duct, which is continuous with the oviduct, the extra-gonadal duct. In the male, the tubule-type testis contains an efferent duct, which functions as the intra-gonadal duct, and a sperm duct extends from the testis, which functions as the extra-gonadal duct. The development of intra-gonadal genital ducts, the ovarian cavity and effer-

ent duct has been investigated in several teleosts (reviewed in Nakamura *et al.*, 1998). However, little is known about the developmental and external opening process of the extra-gonadal ducts, that is, the oviduct and sperm duct.

In medaka *Oryzias latipes*, the oviduct runs from the ovary to an opening in the posterior surface of the urinogenital papillae (UGP) in the adult female (Robinson and Rugh, 1934; Yamamoto and Suzuki, 1955). The UGPs are protuberances between the anus and the genital pore in the female medaka, and represent specific sexual characteristic of the female (Oka, 1931). In the adult male, the sperm duct extends continuously from the efferent duct (Nakamura, 1978) and the end of the sperm duct fuses to the urethra to eventually open up at the surface as the urinogenital pore (Yamamoto and Suzuki, 1955). The developmental process of genital ducts formation has also been previously reported in some aspects (Onitake, 1972; Nakamura, 1978; Kanamori *et al.*, 1985). However, these investigations provided only fragmented descriptions and there was not any sufficient details on the relations to development of inter- and extra-gonadal genital ducts so far. Hence, this study is the first investigation for the entire developmental process of genital ducts in the medaka.

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## MATERIALS AND METHODS

The Hd-rR inbred strain of the medaka, *Oryzias latipes*, was

used for this study. The fish were maintained in aquaria under an artificial photoperiod of 16L:8D at 27±2°C. The fish reached sexual maturation around 90 days after hatching.

Fish between 10 to 90 days after hatching were fixed in Bouin's solution after decapitation, and posterior viscera, including the gonad and genital ductal part were dissected. The total body length of fish described in this study represents the measurement of the fish from the snout to the distal edge of the caudal fin and was measured immediately before fixation. After fixation, specimens were embedded in paraffin and cut serially and either transversely or vertically to the body axis into 5 µm sections. Specimens were stained with hematoxylin and eosin (HE). Some specimens were also stained with periodic acid-Schiff (PAS)-Alcian Blue (AB) since some parts of the genital ducts structure can be visualized more clearly with this method. At least 10 specimens were observed at each stage.

## RESULTS

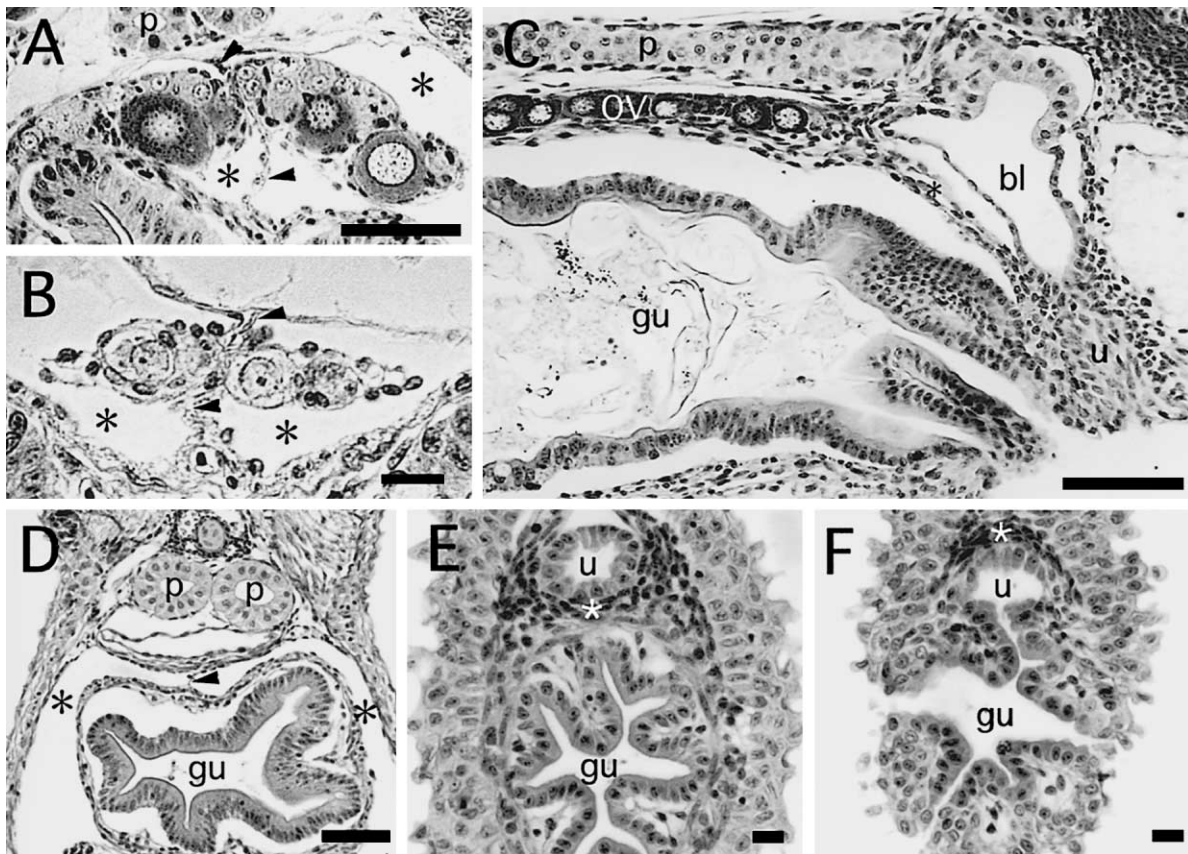
### Before genital duct development (6 to 9mm in total body length, 10 to 20 days after hatching)

Both male and female gonads were bilobed at this stage (Fig. 1A, B). These gonads protruded from a dorsal mesentery (Fig. 1; arrowheads) into the right and left body cavities (Fig. 1; black asterisks). The posterior end of the gonads was adjacent to the anterior part of the bladder (Fig.

1C) but no genital ducts were observed at this stage. Behind the gonads, there was only simple dorsal mesentery made up of epithelia of body cavities (Fig. 1D). In the ventral zone of the urethra, only a supporting layer of the urethra epithelium was seen (Fig. 1C, E; white asterisk). In this stage, the posterior end of the urethra and gut epithelium was fused to form a cloaca (Fig. 1F). There were no differences between the female and male posterior region of the gonads at this stage.

### Development of genital ducts

Initiation of genital duct formation was first identified in both females and males when they had reached a total body length of 8.5 to 10 mm (20 to 30 days after hatching). In this study, we found the association of UGP development and extra-gonadal ducts development. Yamamoto and Suzuki (1955) reported that the UGPs of the medaka consists of two tissues, the cortex and the medulla, and the difference between male and female UGP development was recognizable by observing the external morphology when the fish are more than 16–17 mm in total body length. However, no histological examination has performed in early development of the UGP. Therefore we also noted this process in this study.



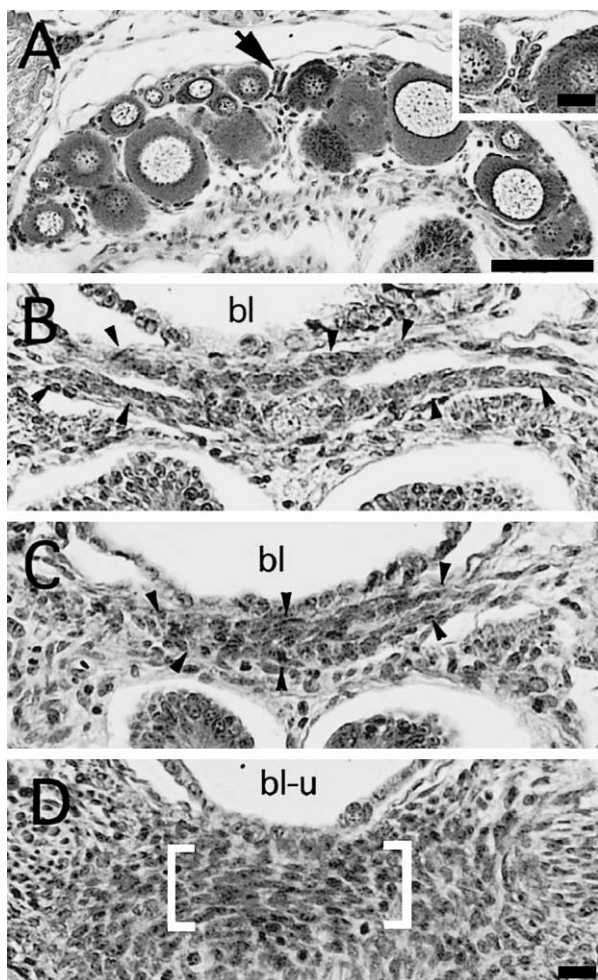
**Fig. 1.** Morphology of the gonad to cloaca region before genital duct development. Transverse section of the gonad in a female (A). Transverse section of the same male at the gonad (B), just behind the gonad (D), at the ventral zone of the urethra (E), and at the cloaca (F) at 10 days after hatching. Vertical section of the posterior part of the trunk in a female 20 days after hatching (C). p: pronephric duct, ov: ovary, gu: gut, bl: bladder, u: urethra. Arrowheads: dorsal mesentery or body cavity epithelium; black asterisks: body cavity; white asterisks: supporting layer of the urethra. (Bars in C: 1 mm; A, D: 50 µm; B, E, F: 10 µm)

## Females

### 1. Appearance of primordial genital ducts (total body length 8.5–10mm, 20–30 days after hatching)

The ovary contained many oocytes at the diplotene stage (Fig. 2 A). At this stage, the right and left parts of the ovary fused and a mass of cells, which sometimes appeared as two layers, was observed at the dorso-central part of the ovary toward the dorsal mesentery (Fig. 2A; arrow and inset).

At the posterior end of the ovary, cell layers began to extend at both the ventral and dorsal sides (Fig. 2B; arrowheads). Behind the ovary, a cell mass was observed (Fig. 2C; arrowheads) and the cell mass contacted with the cell layers at the end of ovary. In the previous stage described



**Fig. 2.** Appearance of primordial genital ducts in a female. Transverse sections of the central part of the ovary (A), the posterior end of the ovary (B), behind the ovary (C) and at the ventral region of the urethra (D) of the same specimen 30 days after hatching (total body length 9.5 to 10 mm). High magnification of the cell mass of the dorso-central part of the ovary is also shown (A, inset). bl: urinary bladder, bl-u: transitional region of urinary bladder to urethra. Arrow: cell mass of dorso-central part of ovary; arrowheads: cell mass of primordial genital ducts; white brackets: thick mesenchyme in the ventral region of the urethra. (Bars in A: 50  $\mu$ m; A inset: 10  $\mu$ m; D: 10  $\mu$ m; B–D are the same magnification.)

above, only the epithelium of body cavities was observed in this region. At the later stages, the cell layers or cell mass differentiated into the ovarian cavity and the oviduct. In this study, we defined a cavity that was observed above the germ cell-containing region (i.e. the region of the ovary) as the ovarian cavity, and the more posterior region of the ovary as the oviduct lumen. In the ventral region of the urethra, mesenchyme cells formed a thicker layer than in the previous stage (Fig. 2D; square brackets).

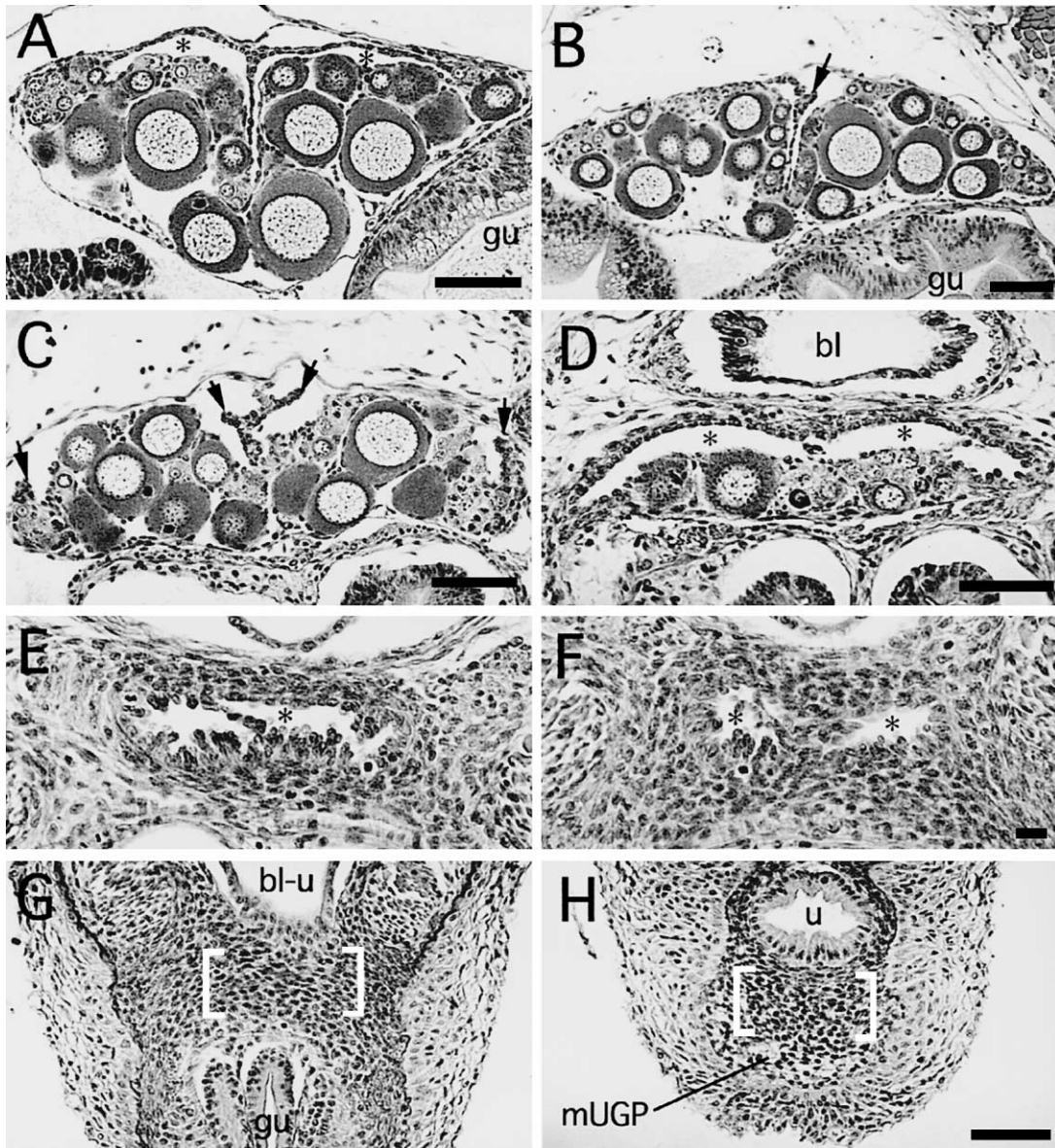
### 2. Development of the ovarian cavity and the oviduct (total body length 10–15mm, 30–50 days after hatching)

The ovarian cavity was observed from the anterior end and posterior end of the ovary respectively (Fig. 3A: anterior; 3D: posterior). The inner surface of the ovarian cavity was lined with a single layer of cuboidal epithelium (Fig. 3A, D). In the part adjacent to these regions, the mass of somatic cells located in the dorso-central part of the ovary had begun to extend laterally, while another mass in the lateral parts of the ovary had begun to extend in a dorso-central direction (Fig. 3C; arrows, the mid posterior region) as previously described by Kanamori *et al* (1985). In mid part of the ovary, only the cell mass located in the dorso-central part of ovary was observed (Fig. 3B, arrow) as in the preceding stage.

At the posterior end of the ovary, a lumen of the oviduct extending from the ovarian cavity was clearly identified at this stage (Fig. 3E, F; asterisks). The oviduct consisted of a rough lining with a single layer of epithelium and of mesenchymal layer. A mass of irregularly shaped cells observed in the lumen of the oviduct (Fig. 3E) was sometimes separated into two lumens at the posterior end of the oviduct (Fig. 3F). At this stage, body cavities were not clearly identified in this region, because several mesenchymal cells were loosely scattered around the oviduct. The oviduct extended continuously from the posterior end of the ovary to the ventral side of the posterior bladder. In the ventral region of the urethra, the layer of mesenchymal cells had further developed and become thicker than in the previous stage (Fig. 3G, H; white square brackets) but there were no cavities in this region. The UGP medulla was first observed at this stage and connected with the mesenchymal cell layer between the anus and urinary pore (Fig. 3H; mUGP). Development of the UGP cortex (epidermis) subsequently separated the cloaca into the anus and urinary pore.

### 3. Formation of the genital pore lip (GPL) (total body length 15–25mm, 40–90 days after hatching)

In this study, we first found at this stage that two layers originated from each side of the UGP cortex had invaginated the ventral region of the urethra mesenchyme and formed cavities during female-specific UGP development. We termed this structure the genital pore lip (GPL). In adults, the GPL cavities became fused to each other and opened up both to the anterior oviduct lumen and the exterior as the posterior part of the oviduct. In the following section, we will



**Fig. 3.** Early development of the ovarian cavity and the oviduct. Transverse sections of the anterior to posterior part of the ovary (A–D), the anterior part (E) and posterior end (F) of the oviduct and the ventral region of the urethra (G, H) of the same fish (total body length 11 to 12.5 mm). bl: urinary bladder, bl-u: urinary bladder to urethra, gu: gut, u: urethra, mUGP: medulla of urinogenital papilla. Asterisks: ovarian cavity (A, D) or lumen of oviduct (E, F); arrows: the cell mass of presumed primordial ovarian cavity; white brackets: the ventral region of the urethra mesenchyme. (Bars in A–D, H: 50  $\mu$ m; F: 10  $\mu$ m; E and F, G and H are the same magnification.)

describe the developmental process of the GPL as three steps.

### 1) Elongation of GPL cortex (total body length 15–20 mm, 40–60 days after hatching)

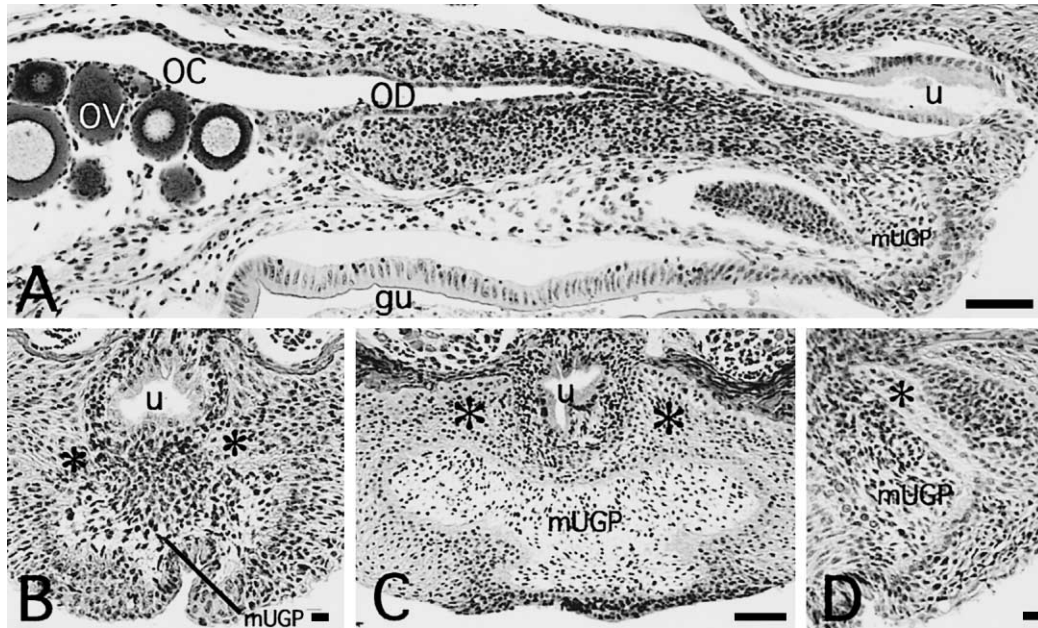
The formation of ovarian cavities was completed and they were noted in the entire ovary at this stage. The anterior oviduct connected to the ovarian cavity consisted of a single layer of the cuboidal epithelium and the outer developing muscular layer (Fig. 4A). However, the posterior end of the oviduct located below the posterior bladder observed in the previous stage had not extended posteriorly (Fig. 4A).

In this stage, the layers, which termed the GPL cortex,

had invaginated the dorsal region of the UGP medulla separately from both the right and left part of the UGP cortex (Fig. 4B; asterisks). As development of the UGP proceeded, right and left GPL cortex became fused to each other at the central part in the dorsal region of the UGP medulla (Fig. 4C; asterisks). At the same time, the GPL cortex had become elongated along the dorsal region of the urethra mesenchyme (Fig. 4D; asterisk).

### 2) Formation of GPL cavities (total body length 20 to 25 mm, 60 to 90 days after hatching)

In this stage, many vitellogenic follicles were observed in some ovaries (Fig. 5A). The supporting layer of the ovi-



**Fig. 4.** Elongation of genital pore lip (GPL) cortex. Vertical sections of midsagittal plane (A) and more lateral plane (D) of the same fish (total body length 20 mm). Transverse sections of the UGP at total body length of 15 mm (B) and 20 mm (C). (B, C) a part of distal end of the gut epithelium was observed below the medulla of urinogenital papilla. OC: ovarian cavity, OV: ovary, OD: oviduct, u: urethra, mUGP: medulla of urinogenital papilla, gu: gut. Asterisks: GPL cortex. (Bars in A–D: 50  $\mu$ m)

duct had developed further and the wall of the oviduct was now clearly observed as a thick muscular tube consisting of three layers: the inner cuboidal epithelium, surrounding mesenchymal and outer muscular layers (Fig. 5B). The posterior end of the oviduct not yet become open to the exterior and still lay below the posterior bladder as in the previous stage (Fig. 5C, J).

The GPL cortex both on the left and right side had extended toward the anterior, and the tip of the cortex had almost reached the posterior end of the anterior oviduct (Fig. 5J). The most striking feature of the GPL cortex at this stage was the cavitation of the GPL cortical tissue (Fig. 5D–J; asterisks). These cavities were formed in the GPL cortex on both sides of urinary pore and extended towards the anterior. In some specimens, outside epidermal cells near the GPL cavity also degenerated (Fig. 5F; arrowhead).

The outer surface of the GPL cortex (epithelia) was more clearly visible with the PAS staining method (Fig. 5 G–J) and these lines were continuous with a PAS positive basement membrane of the UGP epidermis (Fig. 5 H). In addition, the inner surface of the GPL cavity was also faintly stained with PAS (Fig. 5 G–J).

### 3) Female genital duct opening (total body length 25mm or more, 80–90 days after hatching)

In young adult females, the GPL was joined to the anterior oviduct (Fig. 6) and opened to the exterior as a single posterior oviduct (Fig. 6B–D). It was assumed that several steps had occurred simultaneously just before spawning, 1) fusion of the left and right GPL cavities, 2) opening of the GPL cavity to the anterior oviduct at the anterior (proximal) region of GPL and 3) opening up to the exterior in the distal

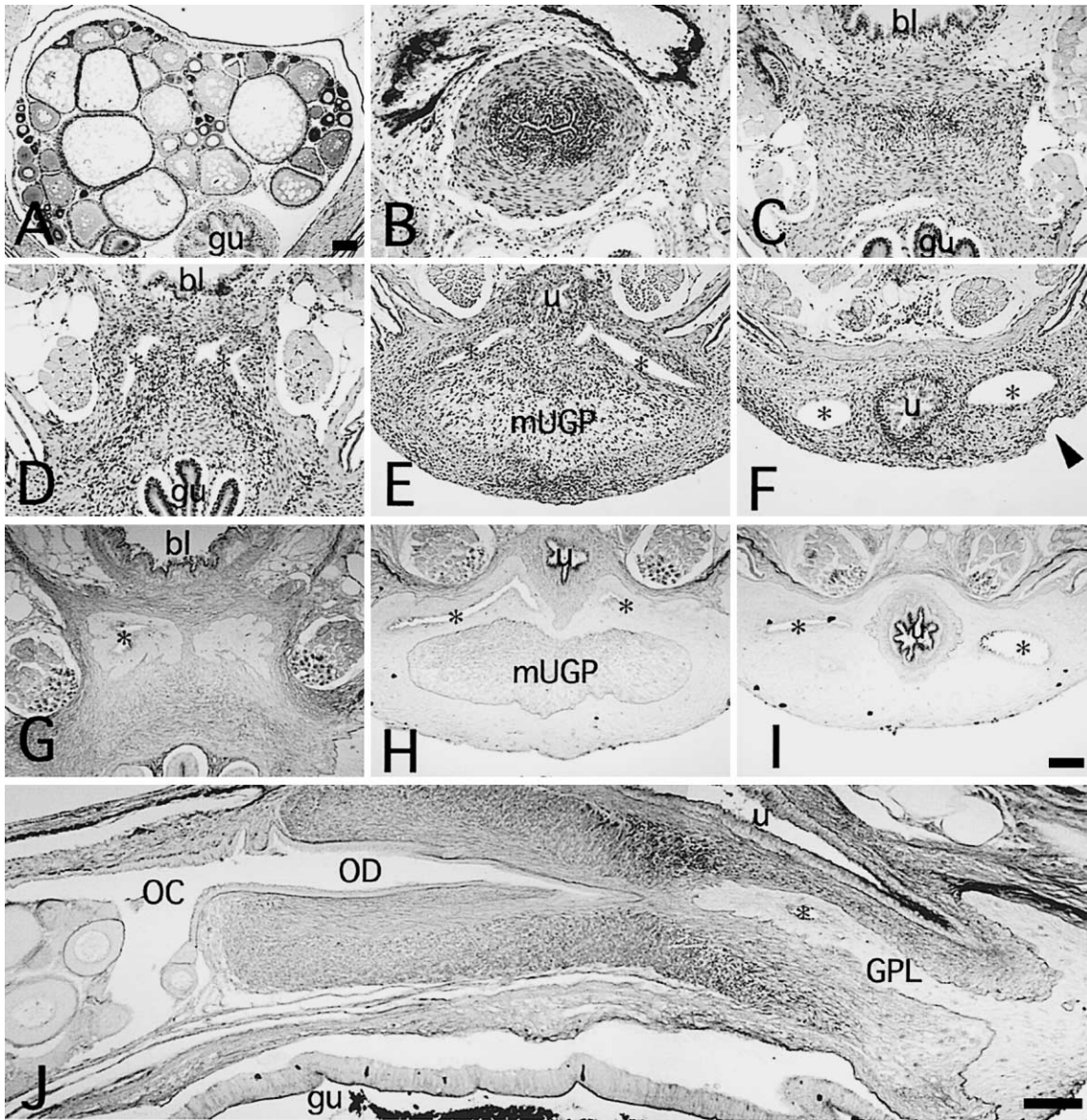
region of the GPL on the UGP medulla.

In this stage, the protuberance of the UGP was more prominent and the posterior end of the UGP had become bilobed or tri-lobed (Fig. 6C). The anterior oviduct was located at the posterior end of the ovarian cavity towards the ventral region of the posterior bladder (Fig. 6D; OD), and the GPL epithelia was located at the posterior end of the anterior oviduct towards the dorsal region of the UGP medulla (Fig. 6D; GPL). The surrounding muscular layer was well developed and supported both the upper oviduct and the anterior region of the GPL (Fig. 6D).

## Male

### 1. Appearance of primordial genital ducts (total body length 8.5–10 mm, 20–30 days after hatching)

In testes, the number of germ cells slightly increased and somatic cells surrounding the germ cell were often observed as presumptive acinous structures (Kanamori *et al.*, 1985). The number of somatic cells was not dominant in the regions containing many germ cells (Fig. 7A). In the posterior end of testes, the number of the somatic cell increased in the central region of gonad to form particularly conspicuous mass (Fig. 7B), and extended between body cavities (Fig. 7C; surrounded by arrowheads). At the later stages, the cell mass differentiated into the efferent duct and the sperm duct. We defined a lumen of that was observed in the germ cell-containing region (i.e. the region of the testis) as the efferent duct, and the more posterior region of the testis as the sperm duct. The ventral region of the urethra mesenchyme had developed further as also observed in the female (Fig. 7D; white square brackets). In this stage, the posterior



**Fig. 5.** Formation of genital pore lip (GPL) cavities. Transverse sections of the same fish with HE staining (A–F) and of another fish with PAS/AB staining (G–J). Both fish were 24 mm in total body length. Vertical section of the ovary to the UGP (J) at a total body length of 25 mm. gu: gut, bl: urinary bladder, u: urethra, mUGP: medulla of urogenital papilla, OC: ovarian cavity, OD: oviduct, GPL: genital pore lip cortex. Asterisks: cavity of genital pore lip cortex, arrowhead: degeneration of body surface. (Bars in A: 100  $\mu$ m; I, J: 50  $\mu$ m; B–I are the same magnification.)

end of the urethra and the gut were partially separated from each other (Fig. 7E).

## 2. Development of efferent duct and sperm duct (total body length 11–15mm, 30–50 days after hatching)

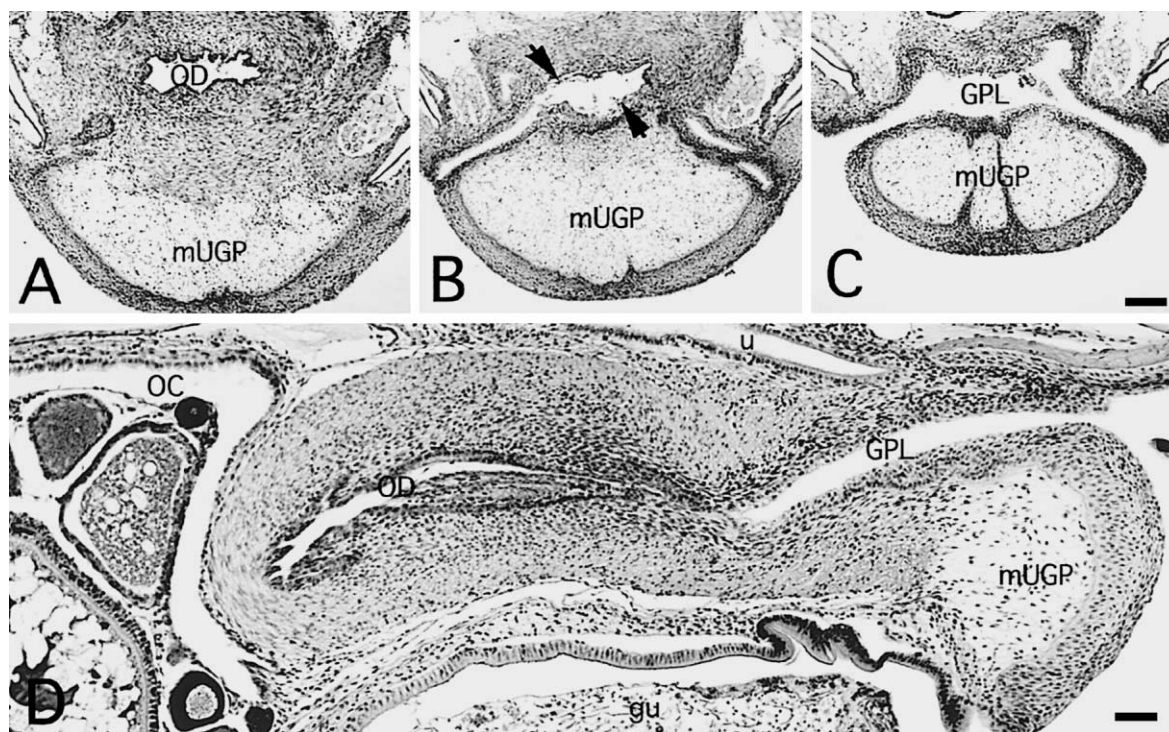
The number of germ cells increased further and the lumen of the efferent duct was clearly observed in the cell mass in the central region (Fig. 7F, G). In the posterior region of the testis, the lumen of the efferent duct was more clearly observed (Fig. 7G) and extended to the lumen of the sperm duct (Fig. 7H). The difference from the female was that body cavities were still distinctly observed around the developing sperm duct at the posterior end of the testis (Fig. 7H).

In the ventral region of the urethra, a cell mass extend-

ing from the sperm duct was observed. Cells in this cell mass were arranged differently from those in the urethra mesenchyme and could be easily distinguished (Fig. 7I; black square brackets). The cell mass adjoined the urethra epithelium near the urinary pore (Fig. 7J; black square brackets). At a later stage, the cell mass developed into an epithelium of the sperm duct in the ventral region of the urethra. In this stage, typical UGP medulla was observed and the posterior end of the urethra and the gut were completely separated as also observed in the female (Fig. 7K).

## 3. Male genital duct opening (total body length 15mm or more, 50–90 days after hatching)

In this stage, spermatogenesis proceeded within each



**Fig. 6.** Female genital duct opening. Transverse sections of the same young adult female with a total body length of 25 mm (A–C) and vertical section of adult female (D). OD: oviduct, ugp: urinogenital papillae, GPL: genital pore lip, OC: ovarian cavity, gu: gut. Arrows: junction between oviduct and GPL epithelium. (Bars in C, D: 100  $\mu$ m; A–C are the same magnification.)

cyst (Fig. 8A) and sperms were observed in the genital duct lumen in some specimens. The efferent duct was lined with a single layer of epithelium and a few connective tissues were observed in the interstitial spaces (Fig. 8B).

In the anterior part of the sperm duct, i.e. the more anterior part of the urethra mesenchyme, the flat sperm duct consisted of a single layer of epithelium supported by a few connective tissues (Fig. 8C). The duct was located between the left and right body cavities (Fig. 8C; asterisks). In the ventral region of the urethra mesenchyme, the folded epithelium of the posterior sperm duct was observed just beneath the urethra epithelium (Fig. 8 D, E). The layer surrounding the posterior sperm duct epithelium was strongly stained by Alcian Blue (Fig. 8I), but the thin layer surrounding the anterior sperm duct was not stained (Fig. 8H). The sperm duct epithelium had become fused to the urethra epithelium near the urinary pore (Fig. 8F, G; arrow). The UGP had not developed and stayed similar to what was seen in the previous stage (Fig. 8D, G).

## DISCUSSION

Detailed studies have been conducted of gonadal development in medaka of both sexes including intra-gonadal genital ducts (Onitake, 1972; Nakamura, 1978; Hamaguchi, 1982; Kanamori *et al.*, 1985). On the other hand, although a few studies have dealt with the formation of the extra-gonadal genital ducts (Onitake, 1972; Nakamura, 1978), no studies have been published which focus

on genital duct development throughout their entire developmental process until their opening to the exterior. This is thus the first description of the entire process of genital duct development.

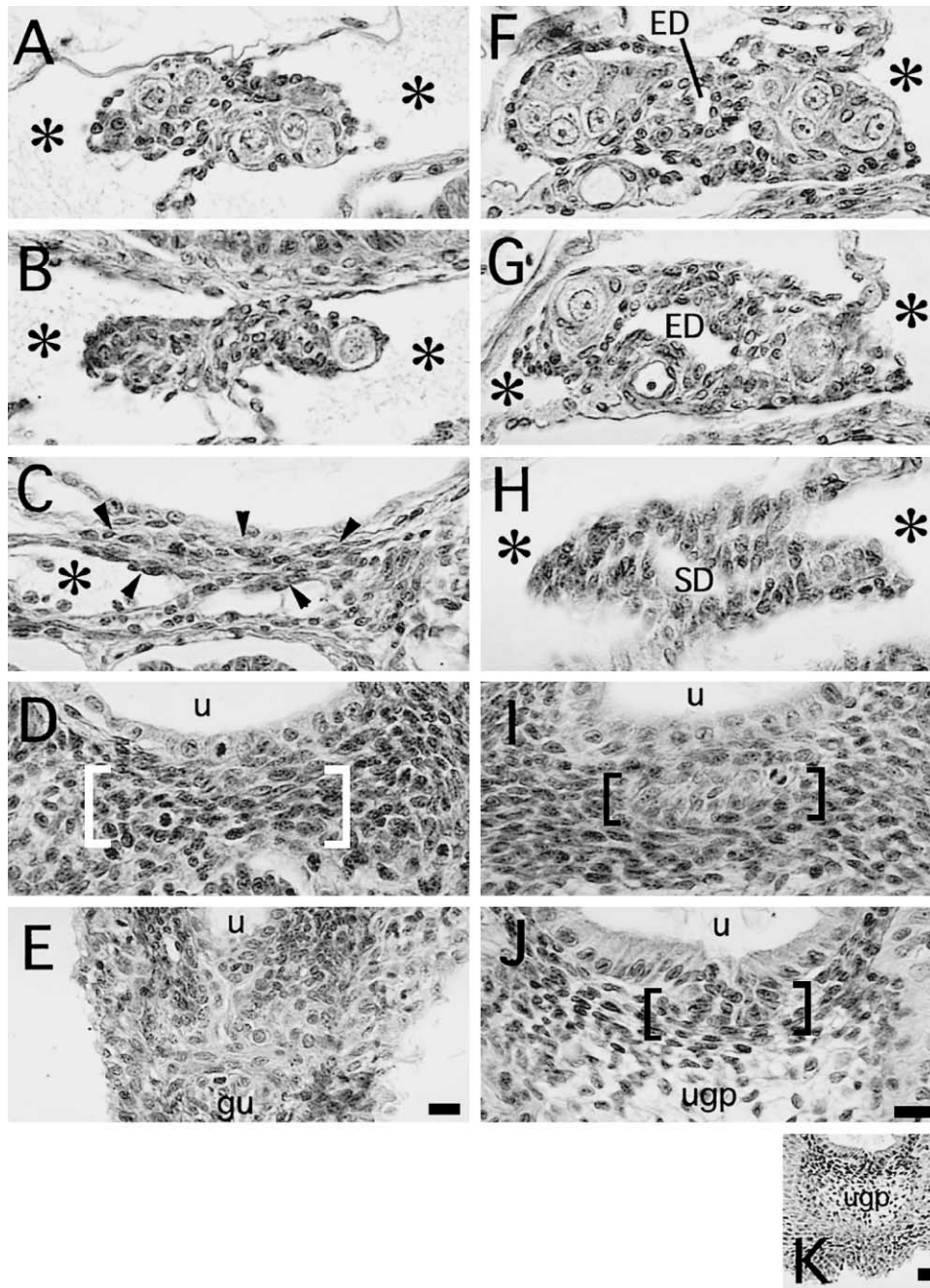
### Extra-gonadal genital ducts contain two parts in both sexes

The present study demonstrated that the anterior and posterior oviduct, the latter termed genital pore lip (GPL) in this study, developed via completely different processes in the female. At the posterior end of the ovary, the anterior oviduct extended continuously from the ovarian cavity to the ventral region of the posterior bladder as described previously (Nakamura, 1978). After formation of the anterior oviduct, the GPL was formed, by means of elongation and cavitation of the cell layers originated from the UGP cortex, in the ventral region of the urethra mesenchyme simultaneously with the female-specific UGP development.

In mammals, the female genital tract, or paramesonephric duct, is not directly open to the exterior. On the other hand, the vaginal plate, which arises from the urinogenital sinus and paramesonephric duct, is formed at the lower part of the genital duct (reviewed in O'Rahilly, 1977). In adults, the vaginal plate becomes the vagina and opens up to the exterior. There is thus an interesting morphological similarity in the development of genital ducts of mammals and medaka, in terms of the formation of new structures for the genital duct opening.

In males, the anterior sperm duct, which is flat and sur-

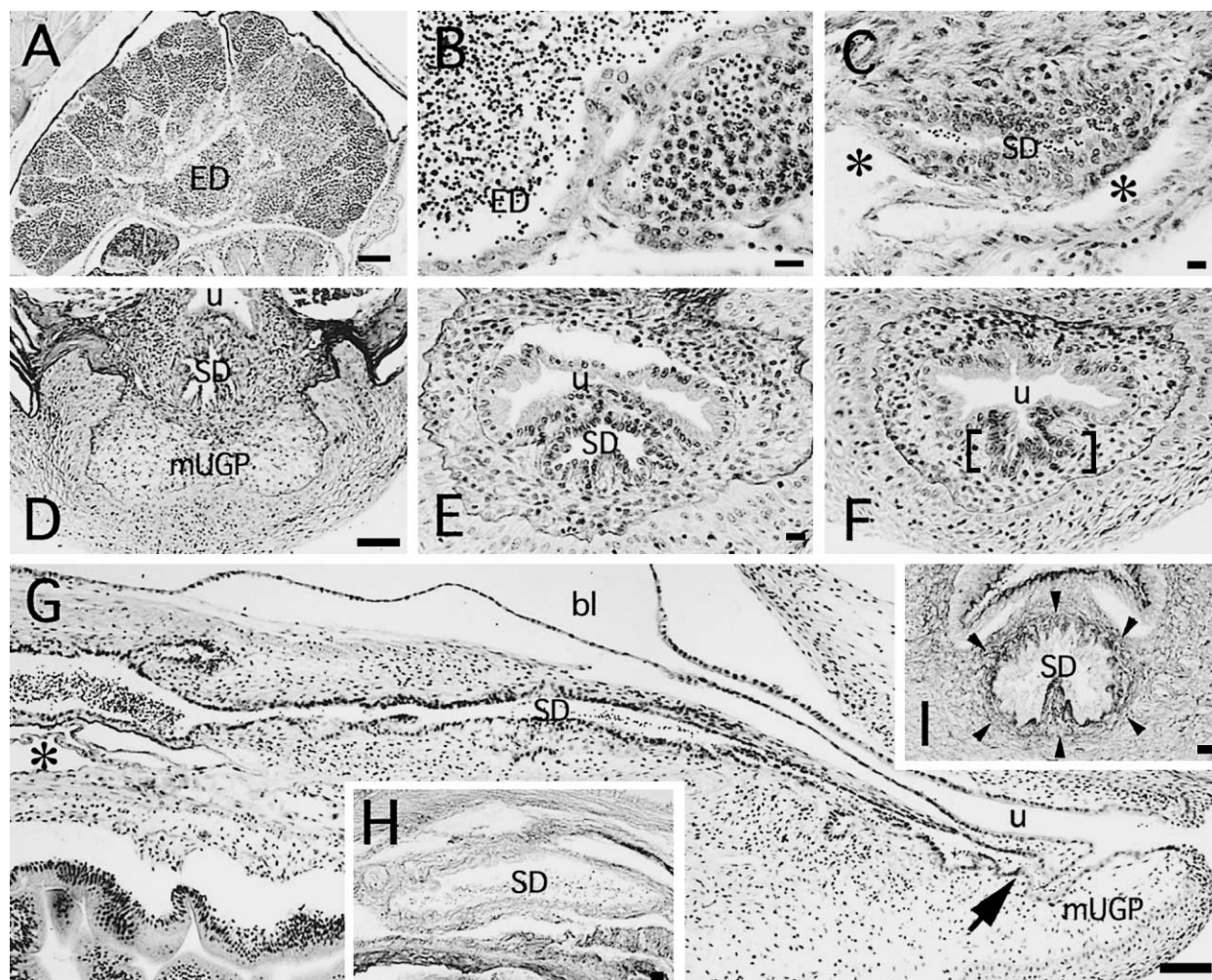




**Fig. 7.** Development of efferent duct and sperm duct. Transverse sections of the testis to cloaca of the same fish with a total body length of 10 mm (A–E) and of 12 mm (F–K). u: urethra, gu: gut, ED: efferent duct, SD: sperm duct, mUGP: medulla of urogenital papilla. Asterisks: body cavity, arrowheads: cell mass of primordial sperm duct, white brackets: urethra mesenchyme in the ventral region, black brackets: sperm duct in the urethra mesenchyme. (Bars in E, J, K: 10  $\mu$ m; A–D and F–J are the same magnification.)

rounded by PAS negative connective tissue, is located between body cavities of the ventral region of the posterior bladder. The posterior sperm duct, on the other hand, which contains the folded epithelium and Alcian Blue positive surrounding layer, is located within the ventral region of the urethra mesenchyme. Although the anterior and posterior parts of the sperm duct in the male were formed continuously from the posterior end of the efferent duct, there are mor-

phological differences between the anterior part and posterior parts of the sperm duct as observed in the female. These findings suggest that extra-gonadal genital ducts comprise two parts, the anterior and posterior ducts, which develop differently in both sexes though the developmental process of the posterior ducts completely differ between female and male.



**Fig. 8.** Male genital duct opening. Transverse sections of the testis to urinogenital pore in the same fish with a total body length of 25 mm (A–F) and vertical section of adult male (G). Transverse sections of another fish stained with PAS/AB (H, I). ED: efferent duct, SD: sperm duct, u: urethra, mUGP: medulla of urinogenital papillae, bl: bladder. Asterisks: body cavity, black brackets: sperm duct, arrow: junction between sperm duct and urethra, arrowheads: layer surrounding posterior sperm duct stained by Alucian Blue. (Bars in A, D, G: 50  $\mu$ m; B, C, E, H, I: 10  $\mu$ m; E and F are the same magnification.)

#### Development of the anterior part of extra-gonadal ducts and intra-gonadal ducts

At a total body length of approximately 8.5–10 mm, a cell mass or layer was observed at the posterior end of gonads in both sexes. At a later stage, the cells of this mass or layer contribute to the formation of the primordia of both intra-gonadal ducts (ovarian cavity or efferent duct) and the anterior part of extra-gonadal ducts (oviduct or sperm duct). These results suggest that the formation of the intra-gonadal ducts and the anterior part of the extra-gonadal ducts are likely to share similar developmental mechanisms. This notion is also supported by observations that an irregularly shaped cell mass, which is a remainder of the cell mass at the dorso-central of the ovary, is sometimes present inside the ovarian cavity (data not shown), and similar irregularly shaped cell masses have also been identified in the oviduct lumen.

In the female, the anterior oviduct primordium did not develop in the ventral region of the urethra mesenchyme. In

contrast, the sperm duct primordium was formed through this region. Because there was no significant difference in the ventral region of the urethra between the sexes, the sexual differences may result from the difference in the primordia between anterior oviduct and sperm duct. It will be of interest to examine the mechanisms of the cell-to-cell interaction between the anterior part of the genital ducts primordia and the urethra mesenchyme.

In this study, we examined intra-gonadal genital duct development with special reference to differences along the anterior-posterior (A-P) axis. In the female, the ovarian cavity formation progressed from the anterior end and posterior end of the ovary after the appearance of the primordial genital duct. However, it is not clear whether there are any relationships between the genital duct formation at the posterior end and at the anterior end of the ovary. The efferent duct formation in the male showed no significant differences along the A-P axis. Thus, one possible explanation of the

differential ovarian cavity formation along the A-P axis is that the cavity formation seems to appear earlier in the peripheral region simply because the region is smaller and has a simpler structure. Other possibility is that ovarian development proceeds exactly from anterior and posterior end of ovary and some factors regulate differential development of the ovary along the A-P axis. Indeed, it has been reported that expression pattern of fibroblast growth factor (FGF) changes along the A-P axis during ovarian development in medaka (Watanabe *et al.*, 1998).

In several fish, sex steroids are also thought to regulate genital duct formation since administration of sex steroids has positive effects on the gonadal ducts (reviewed in Fostier *et al.*, 1983). In medaka, Onikate (1972) reported that administration of estrone induced earlier (anterior) oviduct formation in female, while our previous study demonstrated that the ovarian cavity and anterior oviduct formation was severely inhibited by Fadrozol, which is known to suppress the activity of aromatase, the enzyme catalyzed androgen to estrogen (Suzuki *et al.*, 2004). These results suggest that estrogen must play an important role in the development of female genital ducts in fish.

#### Development of the posterior extra-gonadal genital ducts

Before genital duct development, the urethra mesenchyme consisted of a few cell layers that surround the urethra epithelium. As developmental progressed, only the ventral region of the urethra mesenchyme thickened, and the UGP medulla arose continuously from this region in both sexes. At the later stage, the GPL cortex or the posterior part of the sperm duct was formed in this region. These findings indicate that the development of the ventral region of the urethra mesenchyme is the first step in the lower genital duct formation.

In the female, invagination of the GPL cortex into the urethra mesenchyme occurred as female-specific events after the formation of the anterior oviduct. This remarkable development of the GPL cortex showed good correlation with the UGP development. It has been reported that estrogen treatment induces UGP development in the male (Yamamoto and Suzuki, 1955), and our preliminary result showed that the invagination of the GPL cortex corresponds closely to UGP development in the estrogen treated male. These results suggest that the development of GPL cortex depends on the female specific UGP development and that estrogen must play an important role in these developments. In addition, the presumed opening of the female genital duct just before spawning suggests that other hormones, possibly also involved in oocyte maturation, may play some role(s) in the final development of GPL epithelia. In the male, the posterior part of the sperm duct formed continuously with the anterior part of the sperm duct in the ventral region of the urethra mesenchyme. There are two possible origins of the posterior part of the sperm duct epithelium, the epithelium of the anterior sperm duct or the ventral region of the urethra mesenchyme. If the latter possibility is correct, the different origins of the epithelia may also contribute to

structural difference between the anterior and posterior part of the sperm duct. In addition, surrounding urethra mesenchyme may contribute to the specific morphology of difference the epithelium of posterior sperm duct. Sex steroids may involve in male genital duct development as in the female, although the role of endogenous androgen for the development is not known.

In conclusion, our observations of the development of the posterior part of genital ducts suggest that the ventral region of the urethra mesenchyme plays an important role in the formation of these ducts in both sexes. Further investigation of molecular mechanisms on reproductive tract development is needed since the results may provide some new insights into the development of the reproductive tract in other vertebrates.

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