Immunohistochemical observations of vitellin synthesis and accumulation processes in ovary of Ezo abalone *Haliotis discus hannai*.

Masahiko Awaji, Toshie Matsumoto, Keisuke Yamano, Makiko Kitamura, and Akihiko Hara

Affiliations of the authors

M. Awaji, T. Matsumoto, K. Yamano: National Research Institute of Aquaculture, Minami-Ise, Mie 516-0193, Japan

M. Kitamura, A. Hara: Graduate School of Fisheries Sciences, Hokkaido University, Hakodate, Hokkaido 041-8611, Japan

Corresponding author: Masahiko Awaji, Tel: +81 599 66 1830; fax: +81 599 66 1962

E-mail addresses of the authors: M. Awaji, awajim@affrc.go.jp; T. Matsumoto, mtosie@affrc.go.jp; K. Yamano, yamano@affrc.go.jp; A. Hara, aki@fish.hokudai.ac.jp

Abstract

Ovarian follicle cells are the site of yolk protein synthesis in the Ezo abalone *Haliotis discus hannai*. In this study, histological observations of ovarian follicle cells were conducted in *H. discus hannai* by immunocytochemical and *in situ* hybridization methods focusing on their function of yolk protein synthesis. An antibody raised against purified yolk protein (vitellin, Vn) recognized yolk granules in oocytes, and ovarian follicle cells adjacent to the oocytes under yolk accumulation were stained positively with both anti-Vn antibody and an antisense probe for vitellogenin (the precursor for vitellin, Vtg) mRNA. These results indicate that the abalone Vtg gene is transcribed and translated in the ovarian follicle cells. In oocytes in the early phase of yolk accumulation, positive reactions with the antibody appeared first in the stalk part, and the follicle cells adjacent to the stalk were also stained positively. These observations imply local transportation of yolk protein from the follicle cells to the oocyte through an extracellular space around the oocyte stalk. Ovarian follicle cells changed their morphological characteristics and the reactivity to the antibody with close relationships with the stages of the adjacent oocyte, which suggests the presence of functional interactions between follicle cells and oocytes in the course of yolk protein synthesis and accumulation.

Keywords: Vitellin, Vitellogenin, Follicle cells, Abalone, Mollusk

Introduction

Oocytes of mollusks accumulate yolk protein, vitellin (Vn), during their development. Among mollusks important for fisheries, biochemical analyses of Vn have been conducted in Pacific oyster *Crassostrea gigas* [1], Mexican Pacific oyster *C. corteziensis* [2], scallop *Patinopecten yessoensis* [3,4], Manila clam *Ruditapes philippinarum* [5], softshell clam *Mya arenaria* [6], and Ezo abalone *Haliotis discus hannai* [4]. Purification of the Vn revealed it to be a large molecule ranging from 700 kDa to 450 kDa in its native form and composed of several subunits [1, 3, 4]. The Vn is synthesized as a precursor molecule, vitellogenin (Vtg), and processed by proteases into a mature molecule. The Vtg cDNAs have been clarified in *C. gigas* [7], *P. yessoensis* [8], blue mussel *Mytilus edulis* [9], *H. discus hannai* [10], and in some other mollusks. The amino acid sequences deduced from the cDNAs share 20 to 30 % similarity with Vtg of fish, nematodes, and crustaceans mainly in their N-terminal sequences [7, 10]. Information on molluscan Vtg and Vn obtained from these studies is useful to monitor ovarian growth of the above mentioned species in the wild and under aquaculture conditions [2, 5, 11], and to evaluate the effects of environmental pollutants on reproductive activities of marine and freshwater mollusks [5, 12, 13].

In oysters, scallops, mussels and abalones, oocytes under yolk accumulation are pear-shaped, and attach by their stalks to the basement membrane of a genital tubule in bivalves, and of trabeculae or ovarian cavities in abalone [14, 15]. Ovarian follicle cells are distributed within the germinal

epithelium lining genital tubule or ovarian cavity, and are often observed located around the stalk of oocytes [7, 16]. Vn synthesis has previously been supposed to occur in oocytes in *C. gigas* and *P. yessoensis* [1, 3], but analysis of the Vtg gene expression by *in situ* hybridization has clarified it to occur in the ovarian follicle cells in *C. gigas* [7] and *H. discus hannai* [10], or auxiliary cells (equivalent to the ovarian follicle cells) in *P. yessoensis* [8].

The ovarian follicle cells of oysters, scallops and abalones thus play important roles in the yolk accumulation processes of these species, but histological observations on the follicle cells focusing on their function related to yolk synthesis and accumulation are limited. Changes of the follicle cells in morphology and immunoreactivity to the anti-Vn antibody in relation to oocyte development will provide valuable information to understand processes controlling yolk synthesis and accumulation. The abalone, *H. discus hannai*, is suitable for histological examination of the Vn synthesis of the ovarian follicle cells, since anti-Vn antibody prepared from purified Vn and the cDNA information of the Vtg which exactly codes the purified Vn are both available [4, 10]. In addition, abalones produced in hatcheries and reared at 20 to 22 ^{O}C become sexually mature at around 18 months after metamorphosis (around 50 mm in shell length), and morphological changes of the ovarian follicles cells are clearly observable in these young abalones [16].

In this study, immunohistochemical observations of the follicle cells utilizing anti-Vn antibody were conducted in the abalone *H. discus hannai*. We employed both immunocytochemical and *in*

situ hybridization methods to confirm Vtg gene translation and transcription at the ovarian follicle cells. The processes of yolk synthesis and accumulation were examined by immunocytochemical methods with special emphasis on the relationships between growing oocytes and the associating follicle cells.

Materials and methods

Specimens

Females of *H. discus hannai* (39 to 41 mm in shell length (SL)) produced and reared in a hatchery for 18 months after insemination were used as the materials. The conical appendage of 4 females, composed of digestive diverticula and gonad, was dissected out and fixed with a mixture of saturated mercury chloride and neutral formalin (7:3) for one day at 4 ^{O}C. In addition, the conical appendage of a large female (95 mm in SL) captured from the wild and reared to full maturity in the hatchery was also fixed in the same way. The fixed tissues were rinsed in 70 % ethanol, dehydrated and embedded in paraffin. Horizontal sections were prepared at 3μm thickness, and used for immunohistochemical staining, in situ hybridization and Mayer's hematoxylin and eosin staining. Stages of oocyte development were classified according to the description reported by Tomita [15].

Production of anti-Vn antibody

An anti-Vn antibody used in this study was the one previously produced by one of the authors [4]. Methods for the antibody production are as follows. Abalone Vn was purified from the ovaries using a combination of column chromatography on hydroxylapatite (fast flow type, Nakarai tesque, Kyoto, Japan) and Superose 6 (GE Healthcare Japan, Tokyo, Japan). Antisera to the purified Vn were raised in New Zealand white rabbits by intradermal injections of the antigen emulsified in equal volume of Freund's complete adjuvant. Four boosters of 2 ml of the emulsion were injected subcutaneously into sites along the backs of the rabbits at intervals of 1 week. The blood was collected from the ear vein 1 week after the third booster. An immunoglobulin fraction of the antisera was prepared by precipitation with ammonium sulfate at 40 % saturation. The precipitate was dissolved in 0.01 M phosphate buffer (pH 7.0) containing 0.15 M NaCl (PBS) and dialyzed to the same buffer.

Immunohistochemistry

Deparaffinized sections were treated with 0.2 % I_2 / 0.3 % KI in 70 % ethanol for 10 min followed by rinse in 0.25 % sodium thiosulfate to remove precipitates derived from the fixative. After washing with water, the sections were soaked in 3 % H_2O_2 in methanol for 15 min to eliminate endogenous peroxidase activity, incubated with 3 % normal goat serum (NGS, S-1000, Vector Laboratories Inc., CA, USA) in PBS for 1 h and with anti-Vn antibody (1:4000 dilution with 3 %

NGS in CanGet Signal Solution B (NKB-601, Toyobo Co., Ltd., Osaka, Japan)) for 1 h. The sections were washed with PBS and incubated with a second antibody (Histofine simple stain MAX-PO (MULTI), 424152, Nichirei Biosciences Inc., Tokyo, Japan) for 1 h. After another three washes, the sections were treated with a substrate solution (Histofine simple stain DAB solution, 415171, Nichirei Biosciences Inc.) for about 90 sec to visualize the signal, washed with water and counter-stained with Mayer's hematoxylin for 10 sec. Normal rabbit serum was used as a control. The sections stained with Mayer's hematoxylin and eosin were also prepared for observations on the structure of the abalone gonads.

In situ hybridization

Digoxigenin (DIG)-labeled sense and antisense RNA probes for the abalone Vtg were prepared as described in Matsumoto et al. [10]. Deparaffinized sections were treated with proteinase K (10 μg/ml in 0.1 % tween 20 / PBS, No 9033, Takara Bio Inc., Shiga, Japan) at 37 ^{O}C for 15 min, fixed with 4 % paraformaldehyde (162-16065, Wako, Osaka, Japan) in PBS. Then the sections were preincubated at 42 ^{O}C for 30 min in a prehybridization buffer (50 % formamide, 2x SSC (0.3 M NaCl, 0.33 M Trisodium citrate dehydrate, pH 7.0)), and incubated at the same temperature for 20 h in a hybridization buffer (ISHR7, NipponGene, Tokyo, Japan (50 % formamide, 2x SSC, 1 μg/μl tRNA, 1 μg/μl salmon sperm DNA, 1 μg/μl BSA, 10 % dextransulfate)) containing DIG-labeled

antisense probe at 1 μg/μl. Consecutive tissue sections were stained with DIG-labeled sense probe as a negative control. After the hybridization, the sections were washed with 50 % formamide in 2x SSC, 2x SSC, and 0.2x SSC. Every washing step was conducted twice at 42 ^{O}C for 30 min. The sections were incubated with 4 % Block Ace (UK-B80, DS Pharma Biomedical Inc., Osaka, Japan) in 0.1 % tween 20 / 0.01 M Tris-HCl buffer with 0.15 M NaCl for 30 min and with the Fab fragment of an anti-DIG alkaline phosphatase-conjugated antibody (1:500 dilution, 1093274, Roche Applied Science, Tokyo, Japan) at 4 ^{O}C for 18 h. The signals were visualized with nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate (NBT/BCIP stock solution, 1681451, Roche Applied Science) following the manufacturer's instructions.

Results

Gonads of the 18 months old females contained oocytes in various stages of development up to the secondary yolk globule stage. Oocytes attach to trabeculae with their stalks, and ovarian follicle cells were located adjacent to or near the stalk of the oocytes (Fig. 1). Anti-Vn antibody clearly recognized yolk granules of the oocytes under various stages of yolk accumulation. Ovarian follicle cells adjacent to or near a stalk of the oocytes with yolk granules were also stained positively (Fig. 2a, b). In situ hybridization with an antisense RNA probe for the abalone Vtg mRNA showed

positive signals in the follicle cells showing positive reactions to the anti-Vn antibody (Fig. 2c, d). These results indicate that the abalone Vtg gene is transcribed and translated in the follicle cells.

During oocyte development, shapes of the ovarian follicle cells and their reactivity to the anti-Vn antibody changed greatly. The follicle cells associating chromatin nucleolus stage (Fig. 3a) or oil droplet stage oocytes (Fig. 3b) were discernible by their nuclei being strongly stained with hematoxylin. The cytoplasm of the follicle cells was little, and the positive reaction with the anti-Vn antibody could not be recognized (Fig. 3a, b).

Yolk granules in the cytoplasm of the primary yolk globule stage oocytes were clearly stained with the anti-Vn antibody, and the follicle cells adjacent to the primary yolk globule stage oocytes showed structural changes from the previous stage (Fig. 3c). The nucleus became round (4 to 5 μm in diameter) and chromatin stained with hematoxylin was recognizable in the nucleus. The amount of the cytoplasm increased a little, and the positive reactions with the antibody started to appear in the follicle cells (Fig. 3c). The positive reactions were still difficult to recognize in the follicle cells associating the oocytes at the beginning of this stage (FC2 in Fig. 3e), but the reactions became obvious in accordance with the progress of the yolk accumulation (Fig. 3c). The yolk granules in the oocytes were observed mostly in the stalk especially at the early phase of this stage (Fig. 3c, d).

For the secondary yolk globule stage oocytes, the yolk granules scattered in the cytoplasm among oil droplets were stained positively with the anti-Vn antibody (Fig. 3d, e, f). The follicle cells located

at the stalk of the oocyte became columnar, and a part of the cytoplasm was strongly stained with the anti-Vn antibody (Fig. 3d). The nucleus of the follicle cell was located mostly on the apical side, and the positive reaction with the antibody was found on the basal side. The follicle cells associating the primary and secondary yolk globule stage oocytes were observed to form a monolayer around the stalk of the oocyte (Fig. 3d, e). The surface of the secondary yolk globule stage oocytes besides the stalk part was covered with a jelly coat, 4 to 8 μm in thickness (Fig. 3d, e), and ovarian follicle cells covering the jelly coat were not recognizable. Positive reactions with anti-Vn antibody were not detected in the jelly coat (Fig. 3d, e, f).

The gonad of the large female (95 mm SL) contained many secondary yolk globule stage oocytes, and the oocyte growth proceeded more synchronously compared with the 18 months old females. The ovarian follicle cells associating fully developed oocytes were often very flat and elongated lying between a trabecula and the developed oocytes. The cytoplasm of the follicle cells was recognized to be stained positively with the anti-Vn antibody (Fig. 3f).

In the developing ovaries of the 18 months old abalones, oocytes in the early stages of development could be observed together with oocytes in more advanced stages of development. In such parts of the ovary, the follicle cells in different shapes and reactivity to the antibody were observable very close to each other and histological characteristics of which were closely related to the stages of the oocytes (Fig. 3e). For example, when a secondary yolk globule stage oocyte and an

oil droplet stage oocyte were located adjacent to each other, the follicle cells associating with the secondary yolk globule stage oocyte were columnar with strong reactivity to the antibody (FC3 in Fig. 3e). But in contrast, the follicle cells associating the oil droplet stage oocyte were squamous with a nucleus strongly stained with hematoxylin, and the cytoplasm showed no reaction to the antibody (FC1 in Fig. 3e). The follicle cells associating the oocyte at the beginning of the primary yolk globule stage showed the intermediate state. They possessed round nuclei with chromatin, but the positive reactions with the antibody were still difficult to recognize in the cytoplasm (FC2 in Fig. 3e).

Discussion

The present results clearly showed positive reactions of ovarian follicle cells associated with growing oocytes to anti-Vn antibody in *H. discus hannai*. Positive reactions of the follicle cells were observed both in 18 months old females and a mature female of 95 mm in SL. In the pulmonates *Lymnaea stagnalis* and *Biomphalaria glabrata*, absorption of materials from degenerating oocytes by follicle cells is reported [17]. However in the present study using Ezo abalone, positive signals with anti-Vn antibody and with the antisense Vtg probe were detected simultaneously in the follicle cells. This result shows that the immunoreactivity of the follicle cells does not result from re-absorption of Vn released from degenerating oocytes. These and previous results [10] indicate

that transcription and translation of the Vtg gene occur in the ovarian follicle cells in *H. discus hannai*. Although the antibody used in this study was produced against the purified abalone Vn, there remains a possibility that the anti-Vn antibody also recognize Vtg as reported in other cases [18]. Therefore the antigen(s) immunoreactive to anti-Vn antibody will be described as Vn/Vtg hereafter.

In *H. rufescens* and *H. asinina*, presence of squamous follicle cells covering the oocytes at the outside margin of the jelly coat (peripheral follicle cells in *H. asinina*) is reported based on the observations by electron microscope [19,20]. In the present study, peripheral follicle cells were not discernible and only the ovarian follicle cells at the basal part of the oocyte (basal follicle cells in *H. asinina*) were clearly observed. The peripheral follicle cells may also be present in *H. discus hannai*, but their contribution to vitellogenesis must be low since positive reactions with the anti-Vn antibody were not detected in the jelly coat.

The abalone Vn/Vtg is not detectable in the hemolymph of mature females [4]. This implies that the Vn/Vtg synthesized in the follicle cells is not transported to oocytes via the hemolymph but through the extracellular space between oocytes and associating follicle cells. In the present study, yolk granules started to appear mostly in the stalk part of the oocytes in the early phase of the primary yolk globule stage. The follicle cells around the stalk of these oocytes were positively stained with anti-Vn antibody. These observations imply that the Vn/Vtg produced in the follicle

cells is locally transported to the oocytes through the extracellular space around the oocyte stalk. In the cephalopod *Loligo pealei*, the follicle cell layer surrounding oocytes develop into a follicular syncytium which folds and penetrates into the cytoplasm of the oocytes at the vitellogenic stage [21]. Yolk protein is synthesized in the follicular syncytium and transported into the oocytes through the extracellular space between these two cell types [22]. A similar mechanism of yolk protein transport can be supposed in *H. discus hannai*.

In the ovaries of the 18 months old abalones, oocytes at various stages of development were observed close to each other. Follicle cells formed a monolayer around the stalk of each oocyte, and the morphological characteristics of the follicle cells including the reactivity to the anti-Vn antibody were closely related to the stage of the oocyte. This result suggests functional interactions between oocytes and follicle cells in the processes of Vn synthesis and accumulation. In mollusks, Vn synthesis and accumulation are supposed to be controlled by a hormonal substance, such as estrogen in bivalves [23, 24], dorsal body hormone in pulmonates [25], and optic gland gonadotropin in cephalopods [26]. In addition to these hormonal controls, the present observations on the difference of vitellogenic activities in follicle cells imply that as yet undetermined interactions between oocytes and the associating follicle cells might be involved in the control mechanisms of the Vn synthesis in mollusks. For example, the responsiveness of follicle cells to a hormonal factor for Vn/Vtg synthesis might develop through interactions with the adjacent oocyte at the oil droplet stage. Such

mechanisms might be necessary for the occurrence of Vn/Vtg synthesis in the follicle cells in concert with the development of the adjacent oocyte.

Acknowledgements

The authors express their sincere gratitude to Dr. T. Kobayashi of the Iwate Fisheries Technology Center for supplying the abalone samples for this study. This work was supported by a grant from the Fisheries Research Agency.

References

1. Suzuki T, Hara A, Yamaguchi K, Mori K (1992) Purification and immunolocalization of a vitellin-like protein from the Pacific oyster *Crassostrea gigas*. Mar Biol 113: 239-245

2. Arcos FG, Ibarra AM, Rodriguez-Jaramillo MdC, Garcia-Latorre EA, Vazquez-Boucard C (2009) Quantification of vitellin/vitellogenin-like proteins in the oyster *Crassostrea corteziensis* (Hertlein 1951) as a tool to predict the degree of gonad maturity. Aquaculture Res 40: 644-655

3. Osada M, Unuma T, Mori K (1992) Purification and characterization of a yolk protein from the scallop ovary. Nippon Suisan Gakkaishi 58: 2283-2289

4. Kitamura M (2000) Immunochemical and biochemical studies on egg yolk protein, vitellin, in Ezo abalone (*Haliotis discus hannai*). PhD dissertation, Hokkaido University, pp. 1-125, Hakodate

5. Park KI and Choi KS (2004) Application of enzyme-linked immunosorbent assay for studying of reproduction in the Manila clam *Ruditapes philippinarum* (Mollusca: Bivalvia). I. Quantifying eggs. Aquaculture 241: 667-687

6. Gagne F, Blaise C, Pellerin J, Gauthier-Clerc S (2002) Alteration of the biochemical properties of female gonads and vitellins in the clam *Mya arenaria* at contaminated sites in the Saguenay Fjord. Mar Environ Res 53: 295-310

7. Mastumoto T, Nakamura AM, Mori K, Kayano T (2003) Molecular characterization of a cDNA encoding putative vitellogenin from the Pacific oyster *Crassostrea gigas*. Zool Sci 20: 37-42

8. Osada M, Harata M, Kishida M, Kijima A (2004) Molecular cloning and expression analysis of vitellogenin in scallop, *Patinopecten yessoensis* (Bivalvia, Mollusca). Mol Reprod Develop 67: 273-281

9. Puinean AM, Rotchell JM (2006) Vitellogenin gene expression as a biomarker of endocrine disruption in the invertebrate, *Mytilus edulis*. Mar Environ Res 62: S211-S214

10. Matsumoto T, Yamano K, Kitamura M, Hara A (2008) Ovarian follicle cells are the site of vitellogenin synthesis in the Pacific abalone *Haliotis discus hannai*. Comp Biochem Physiol A 149: 293-298

11. Baba K, Miyazono A, Hara A (2004) Relationship between the vitellin concentration in the hemolymph and oocyte necroses during the annual reproduction cycle in the Japanese scallop,

Mizuhopecten yessoensis. Invert Reprod Develop 45: 175-184

12. Puinean AM, Labadie P, Hill EM, Osada M, Kishida M, Nakao R, Novillo A, Callard IP, Rotchell JM (2006) Laboratory exposure to 17β-etradiol fails to induce vitellogenin and estrogen receptor gene expression in the marine invertebrate *Mytilus edulis*. Aquat Toxicol 79: 376-383
13. Won SJ, Novillo A, Custodia N, Rie MT, Fitzgerald K, Osada M, Callard IP (2005) The freshwater mussel (*Elliptio complanata*) as a sentinel species: Vitellogenin and steroid receptors. 2005. Integr Comp Biol 45: 72-80
14. Galtsoff PS (1964) Organs of reproduction. In: The American oyster *Crassostrea virginica* Gmelin. Fish. Bull. Fish Wildlife Service. Vol 64. United States Government Printing Office, Washington, pp 297-323
15. Tomita K (1967) The maturation of the ovaries of the abalone, *Haliotis discus hannai* Ino, in Rebun Island, Hokkaido, Japan. Sci Rep Hokkaido Fish Exp Stat 7: 1-7 (in Japanese with English abstract)
16. Awaji M, Hamano K (2004) Gonad formation, sex differentiation and gonad maturation processes in artificially produced juveniles of the abalone, *Haliotis discus hannai*. Aquaculture 239: 397-411
17. Jong-Brink M de, Geraerts PM (1982) Oogenesis in gastropods. Malacologia 22: 145-149
18. Tsukimura B, Waddy SL, Vogel JM, Linder CJ, Bauer DK, Borst DW (2002) Characterization

and quantification of yolk proteins in the lobster, *Homarus americanus*. J Exp Zool 292: 367-375

19. Martin GG, Romero K, Miller-Walker C (1983) Fine structure of the ovary in the red abalone *Haliotis rufescens* (Mollusca: Gastropoda). Zoomorphol 103: 89-102

20. Apisawetakan S, Linthong V, Wanichanon C, Panasophonkul S, Meepool A, Kruatrachue M, Upatham ES, Pumthong T, Sobhon P (2001) Ultrastructure of female germ cells in *Haliotis asinina* Linnaeus. Invert Reprod Develop 39: 67-79

21. Selman K, Arnold JM (1977) An ultrastructural and cytochemical analysis of oogenesis in the squid, *Loligo pealei*. J Morph 152: 381-400

22. Selman K, Wallace RA (1978) An autoradiographic study of vitellogenesis in the squid, *Loligo pealei*. Tissue & Cell 10: 599-608

23. Li Q, Osada M, Suzuki T, Mori K (1998) Changes in vitellin during oogenesis and effects of estradiol-17β on vitellogenesis in the Pacific oyster *Crassostrea gigas*. Invert Reprod Develop 33: 87-93

24. Osada M, Takamura T, Sato H, Mori K (2003) Vitellogenin synthesis in the ovary of scallop, *Patinopecten yessoensis*: Control by estradiol-17β and the central nervous system. J Exp Zool 299A: 172-179

25. Geraerts PM and Joosse J (1975) Control of vitellogenesis and of growth of female accessory sex organs by the dorsal body hormone (DBH) in the hermaphroditic freshwater snail *Lymnaea*

stagnalis. Gen Comp Endocrinol 27: 450-464

26. O'Dor RK, Wells MJ (1975) Control of yolk protein synthesis by *Octopus* gonadotropin *in vivo* and *in vitro* (Effects of *Octopus* gonadotropin). Gen Comp Endocrinol 27: 129-135

Figure captions

Fig. 1 Histological structure of an ovary of the Ezo abalone. a, Oocytes (OC) adhere to a trabeculae (T) by their stalks (arrowheads). Scale, 200 μm. b, Pear-shaped oocytes (OC) on a trabeculae (T) and ovarian follicle cells (arrowheads) near the stalk of the oocytes. JC, jelly coat. Scale, 50 μm

Fig. 2 Immunohistochemical detection with anti-vitellin (Vn) antibody and *in situ* hybridization of vitellogenin (Vtg) mRNA in an ovary of the Ezo abalone. Ovary sections stained with anti-Vn antibody (a, d) show presence of Vn or Vtg (stained dark gray in black and white photo, or brown in color photo) in oocytes (OC) and ovarian follicle cells (FC). Staining of adjacent sections with an antisense probe for Vtg mRNA (c, stained dark gray in black and white photo, or purple in color photo) and anti-Vn antibody (d) indicates presence of Vtg mRNA and Vn or Vtg in the same ovarian follicle cells (arrowheads). Control staining was conducted in adjacent sections with normal rabbit serum (b) and a sense probe (inset of c). JC, jelly coat. Scale, 50 μm

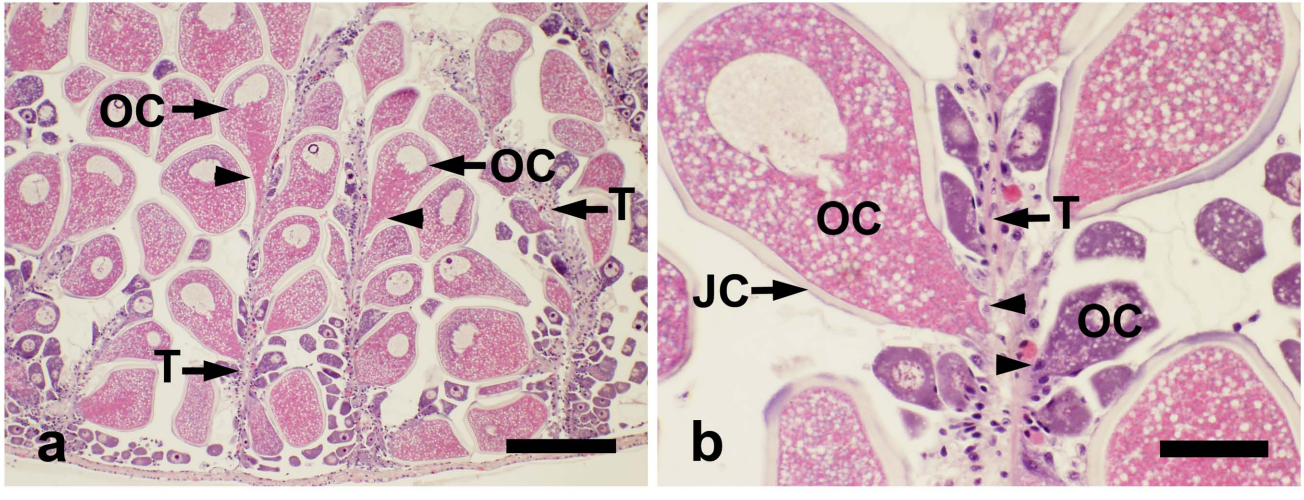
Fig. 3 Changes in vitellin synthesis activity of ovarian follicle cells in relation to the yolk accumulation processes of oocytes. Oocytes (OC) at chromatin nucleolus (a), oil droplet (b), primary yolk globule (c, d), secondary yolk globule (d, e, f) stages and associating follicle cells (arrowheads) were examined with anti-vitellin (Vn) antibody. Vn or vitellogenin synthesis in the follicle cells indicated by the positive reaction with the antibody (stained in brown) progresses in concert with the

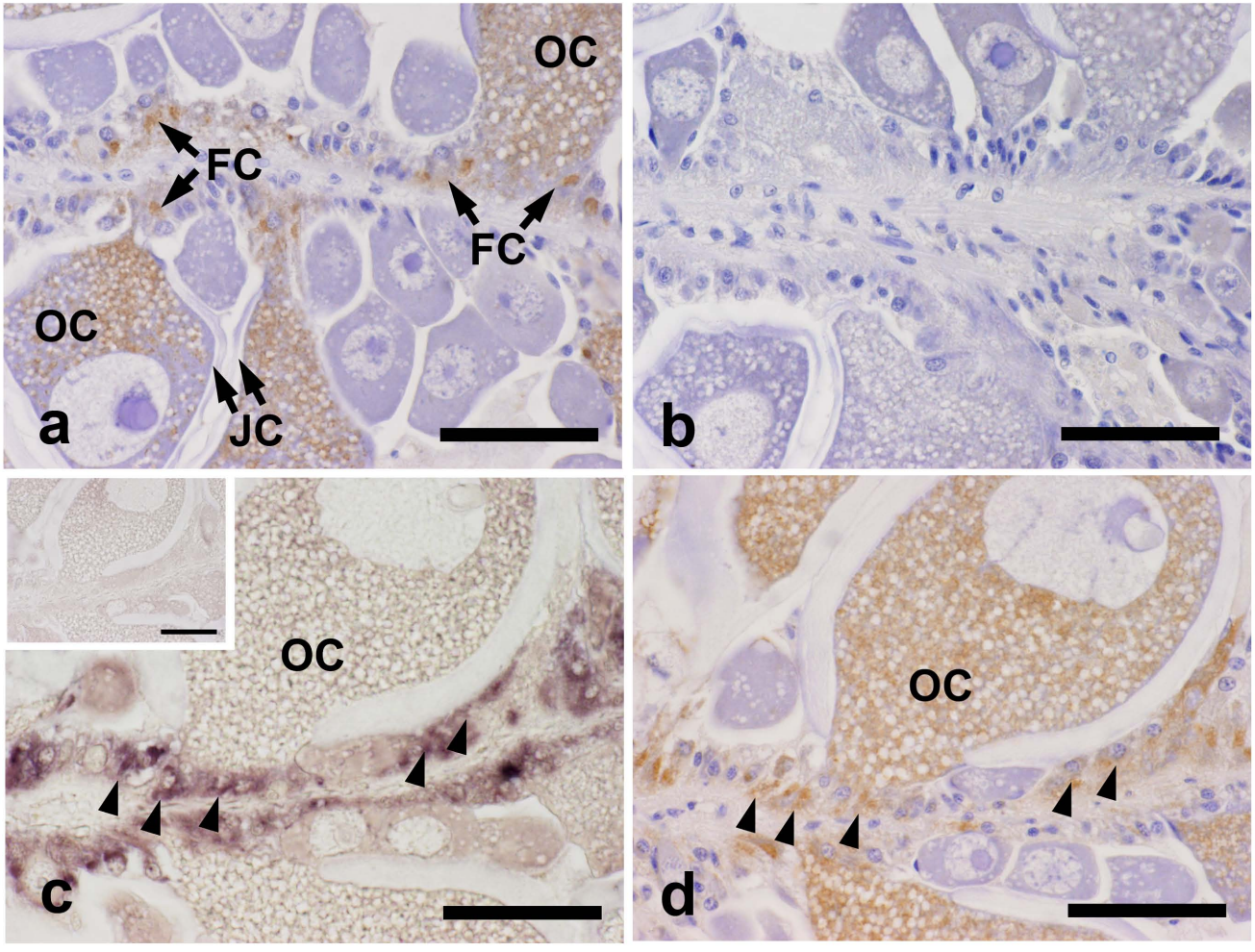
development of the adjacent oocyte. An inset of c, enlargement of the follicle cells; an arrow in b, oil droplet; DD, digestive diverticula; JC, jelly coat; OG, oogonia; YG, yolk granules. Scale, 50 μm

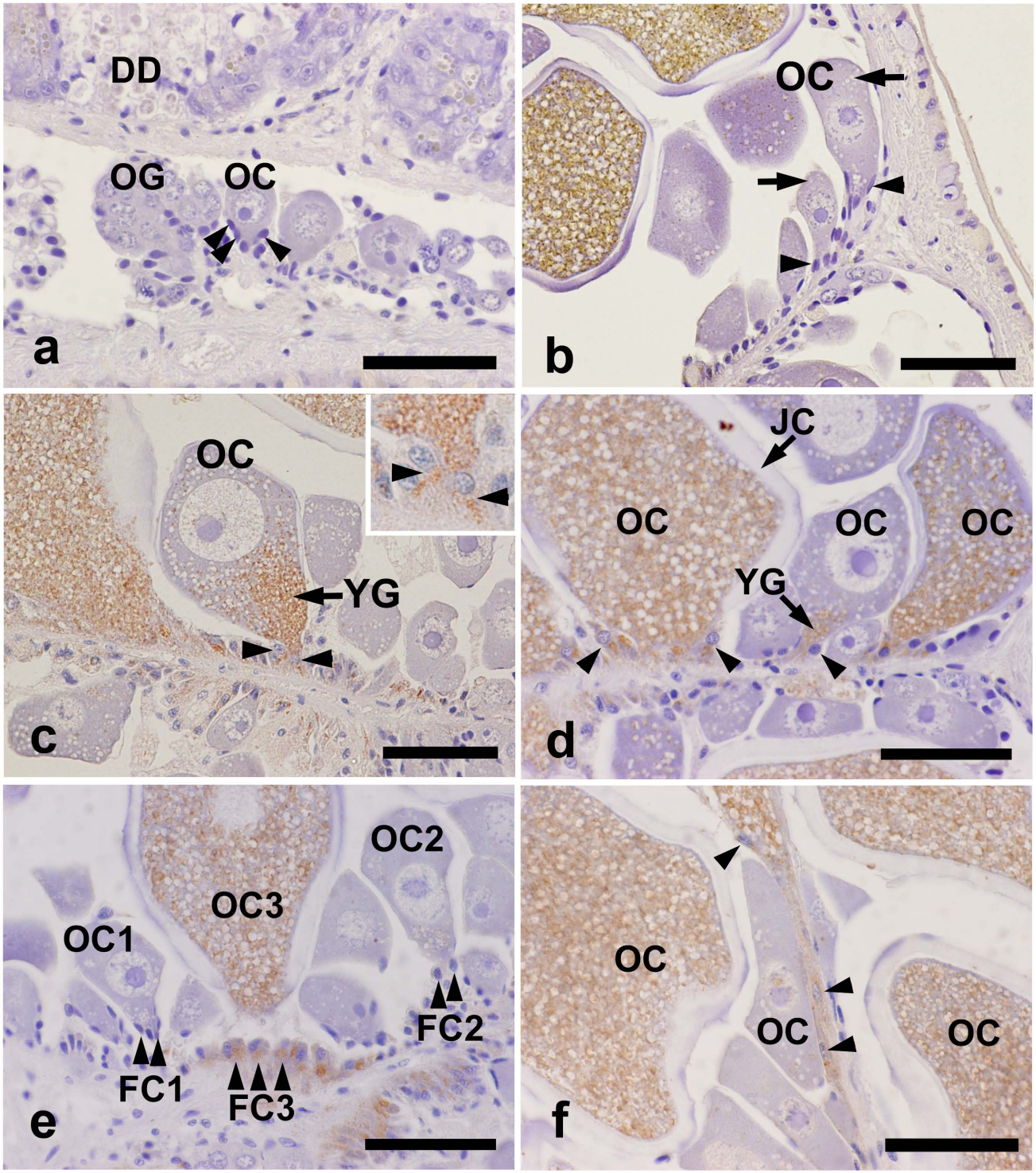
エゾアワビ卵巣におけるビテリンの産生と蓄積過程の免疫組織学的解析

淡路雅彦、松本才絵、山野恵祐（水研セ養殖研）、北村真紀子、原　彰彦（北大院水）

エゾアワビ卵巣濾胞細胞（濾胞細胞）の卵黄タンパク質（ビテリン、Ｖｎ）合成と卵母細胞へのＶｎ蓄積過程について、抗Ｖｎ抗体による免疫染色とＶｎ前駆体遺伝子の in situ ハイブリダイゼーションで組織学的に検討した。その結果、前駆体遺伝子が濾胞細胞で転写、翻訳されることが示され、合成された卵黄タンパク質は濾胞細胞と卵母細胞が接する卵柄部で卵母細胞内に輸送されると示唆された。また濾胞細胞の形態と抗Ｖｎ抗体への反応性は隣接する卵母細胞の発達と密接に関連し、Ｖｎ合成と蓄積において濾胞細胞と卵母細胞間に相互作用が存在すると推定された。


OC
OC
T
T
a
OC
T
JC
OC
b


OC
FC
FC
OC
a
JC
b
OC
c
OC
d


DD
OG
OC
a
OC
b
OC
YG
c
JC
OC
OC
OC
YG
d
OC2
OC3
OC1
FC2
FC1
FC3
e
OC
OC
OC
f