An Unusual Architecture of Occluding Junctions between Surface Cells in Teleost Ovarian Follicles (Plecoglossus altivelis)*

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Summary. An unusual architecture of occluding junctions between surface cells in teleost ovarian follicles was observed by electron microscopy, by using the freeze-fracture technique. On the P-face, double strands of intramembraneous particles are observed. A narrow furrow-like gap is recognized between two rows of particles in such double strands. On the E-face, two types of grooves can be distinguished. Type I groove consists of a wide furrow. Type II groove, which appears to be sequent to type I groove, consists of a pair of "sub-grooves" running parallel. Further, a row of particles appears to be located in the bottom of type I groove or between two "sub-grooves" of type II groove. These observations suggest that the double strands on the P-face are registered with the grooves (type I or type II) on the complementary E-face and that a row of particles on the E-face is registered with a furrow-like region between two rows in the double strands on the P-face. In other words, a single unit of the juncture of the present occluding junction is thought to consist of triplicated junctional strands.

Unusual architectures of occluding junctions have been reported in previous studies. On the P-face, not only grooves but also double linear arrangements of intramembraneous particles have been shown by Claude and Goodenough (1973) and Staehelin (1973), and by Nagano et al. (1977). On the other hand, the strand preferentially located on the E-face has been described by several investigators (Simionescu et al., 1975, 1976; Gilula et al., 1976; Nagano and Suzuki, 1976a, 1976b; Nagano et al., 1977). However, there seem to be no reports concerning the particular area which displays simultaneously both double strands of intramembraneous particles on the P-face and single strands at the bottom of a wide groove or between two narrow grooves on the complementary E-face.

This paper will describe such an unusual architecture of occluding junctions between surface cells in teleost ovarian follicles by using the freeze-fracture technique.

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MATERIAL AND METHOD

About thirty fish of Plecoglossus altivelis, an annual water river fish, were collected in August or September at the Aya Fisheries Experiment Station (Miyazaki Prefecture). Ovaries dissected from the fish were placed in 2.5% glutaraldehyde buffered by cacodylate buffer (pH 7.2) for 2–4 hrs for the freeze-fracture method. The materials fixed by glutaraldehyde were then immersed in 30% glycerine overnight in a refrigerator. The materials were mounted on specimen holders and frozen in liquid Freon 22 cooled with liquid nitrogen. Specimens were cleaved at $-110^\circ$C and the fractured surface contours were replicated by platinum-palladium followed by carbon in $10^{-6}$ Torr in a freeze-fracture device (HFZ-1, Hitachi company). Organic substances on the replica were digested in both disodium hypochlorite (bleach) and chromic acid mixture, and then the replicas were washed in distilled water. The replicas on a 300–400 mesh copper grid were observed with a JEOL 100B and a Hitachi H–300 electron microscopes. Some ovaries dissected from the fish were used for the conventional thinsectioning method. The pre-fixed ovaries with 2.5% glutaraldehyde buffered by cacodylate buffer (pH 7.2) were placed in 2% osmium tetroxide in the same buffer for 2 hrs. They were then dehydrated in a series of ethanol followed by 2 changes in propylen oxide. The specimens were embedded in Epon 812. Thin sections cut on a Porter-Blum MT-2B ultratome with a diamond knife were stained by uranyl acetate and lead citrate and then examined with the same electron microscope mentioned above.

RESULTS

The outermost covering cells in teleost ovarian follicles are conveniently termed "surface cells" in this paper, since the cells have not been suitably named so far. The surface cells are clearly identified by the particular location and the squamous contour (Fig. 1). The so-called strands and

![Fig. 1. A part of a follicle including the oocyte (O), chorion (C), attaching layer (A), follicle cells (FC), basal lamina (BL), thecal cells (TC) and surface cells (SC). Arrows indicate closely apposed membranes between the surface cells (SC). ×12,000](image)
grooves are displayed simultaneously on the P-face and the E-face of the plasma membrane of the surface cells (Fig. 2).

On the P-face: Double strands of intramembraneous particles are frequently observed (Fig. 2-5). Such strands consist of two rows of rather irregular-shaped, low particles (Fig. 3-5). Double strands are less anastomosed and have simple architectures such as a snaky line (Fig. 3), a simple linear one (Fig. 4) and a parallel one of double strands (Fig. 5). A particle-free, furrow-like region can be observed between two rows of low particles in each of the double strands (Fig. 3-5). Tall particles are haphazardly scattered in some areas in such strands. The double strands have a considerable width, being rather narrower than the width of triple particles (Fig. 3-5). Discrete short strands consisting of irregular-shaped particles sometimes lie on one or the other side directly adjacent to a linear strand of regular-shaped, tall particles (Fig. 6).

On the E-face: Less developed anastomosing networks consisting of rows of intramembraneous particles are frequently observed (Fig. 7-9). In some areas, a row of particles appears to be located in the bottom of a wide groove, or runs along linear

![Fig. 2. Low power electron micrograph showing part of surface cells. Double strands of particles (asterisk) on the P-face (P) and single strands in grooves (arrows) on the E-face (E). × 20,000](image)
Fig. 3. Snaky double strands of low particles on the P-face (P). A linear particle-free region between two rows of particles is visible. ×78,000

Fig. 4. Linear double strands on the P-face (P). A linear particle-free region is also visible. ×75,000

Fig. 5. Parallel double strands on the P-face (P). ×75,000

Fig. 6. A single linear arrangement of tall particles (arrows) and a short double strand of low particles (asterisk), both on the P-face (P). Discrete short strands of low particles run close to a single strand of tall particles (double arrows). ×75,000
furrow-like regions (Fig. 7, 8). Moreover, two types of grooves were discriminated on the basis of the morphology. Type I groove consists of a wide furrow, which is apparently wider than the width of a single particle, whereas it is rather narrower than the width of triple particles (Fig. 7, 8). Type II groove consists of a pair of

Fig. 7. Strands and grooves on the E-face (E). A row of particles appears to be located at the bottom of a wide groove (arrowheads). A few rows of particles run along narrow grooves (arrows). $\times 67,000$

Fig. 8. Wide type I grooves ($I_g$) on the E-face (E) and a double strand (asterisk) on the P-face (P). A double strand appears to be sequent to a wide groove in both sides of the fracture (double arrow). Rows of particles are located at the bottom of some grooves (arrowheads). $\times 78,000$
narrow furrows (Fig. 9). For convenience we term each furrow of a pair of narrow ones a "sub-groove" in this paper, in contrast to a wide groove (type I groove). Such two "sub-grooves" run parallel to each other, being divided by a gap about the width of a single particle. A strand of regular-shaped particles appears to be located between such two "sub-grooves" in some areas. The total width of a pair of "sub-grooves" is roughly equal to that of type I grooves, while each "sub-groove" is rather narrower than the width of a single particle. Further, the width of type I or type II grooves is roughly equal to the double strands on the E-face.

DISCUSSION

The present findings provide new information about the architecture of occluding junctions. A particle-free region between two rows of particles in the double strands in this study seems to be much wider than that reported by NAGANO et al. (1977). In contrast to the usual grooves in previous investigations (WADE and KARNOVSKY, 1974; SIMIONESCU et al., 1975, 1976; GILULA et al., 1976; NAGANO and SUZUKI, 1976a, 1976b; NAGANO et al., 1977), the structures reported in the present study deserve attention in two points: First, the width of type I or type II grooves in this study appears to be wider, and second, type II groove shown in Figure 8 is clearly distinguished into two "sub-grooves" and in some areas a strand of particles appears to be located between them. These morphological characteristics seem to have been reported by none of the previous authors.

The observations in this study suggest that double strands on the P-face are registered with a wide groove (type I) or a pair of "sub-grooves" (type II) on the E-face; each row of the double strands is registered respectively with each "sub-groove" of type II groove, and that a strand of particles in the bottom of the wide type I groove or between two "sub-grooves" of type II groove on the E-face is registered with a furrow-like region between two rows of particles of the double strand on the P-face; a furrow-like region may be homologous to a groove itself. Therefore, a single unit of the juncture of the present occluding junction is supposed to consist
of triplets of the junctional strands. Type II groove may be a well clarified feature of such an occluding junction, while type I groove may be an unclarified one probably due to technical problems in the process of making replicas.

In the point of adhering or sealing capacity, a unit consisting of triplicated junctional strands may be stronger than a usual unit consisting of a paired junctional strand, because of its sealing capacity of lanthanum tracer in spite of their less developed anastomosis (Toshimori, Yasuzumi and Oura, unpublished data).

On the other hand, a question is now raised as to why several short strands of irregular-shaped, low particles lie in close relation to a strand of regular-shaped, tall particles on the P-face as shown in Figure 5. The answer is still unknown.

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REFERENCES


