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Short note/Kurze Mitteilung

Tight and gap junctions in the follicle epithelium of vitellogenic oocytes in the Least killifish, *Heterandria formosa* (Poeciliidae)

Zonulae occludentes und nexus im Follikelepithel vitellogenetischer Oocyten des Zwergkärpflings, *Heterandria formosa* (Poeciliidae)

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Zusammenfassung: Wir belegen anhand von Gefrierbrüchen nexus (gap junctions) und zonulae occludentes (tight junctions) im Follikelepithel vitellogenetischer Oocyten nulliparer Weibchen des Lebendgebärenden Zahnkarpfens *Heterandria formosa*. Das bedeutet, dass beide Typen von Zell-Zellverbindungen bereits vor der Besamung und Befruchtung und nicht, wie postuliert wurde, erst nach Bildung der Zygote vorhanden sind. Darüber hinaus enthalten die Mikrovilli der Oocyte Aggregationen von Partikeln in einer Größe, wie sie auch in gap junctions vorkommen.

Poeciliids are viviparous teleosts exhibiting follicular gestation, i.e. the embryo completely develops in the follicle and birth coincidences with ovulation. To allow exchange between the mother and the offspring a follicular placenta has been evolved in poeciliids consisting of the follicular wall (follicle epithelium, underlying blood vessels and theca) as maternal component and the embryonic surface, particularly pericardial sac and yolk sac as embryonic component. Both are separated by a thin modified zona pellucida until parturition. Transfer of nutrients to the embryo via the follicle epithelium varies between species leading to the distinction of lecithotrophic species (solely yolk depending) and matrotrophic species (after a short lecithotrophic phase nutrition by the female) (for review see WOURMS et al 1989; GREVEN, in press).

Hitherto only two poeciliid placentae have been studied by transmission electron microscopy representing the two endpoints of the continuum, i.e. the lecithotrophic guppy *Poecilia reticulata* (JOLLIE & JOLLIE 1964a, b) and the highly matrotrophic and superfetating Least killifish *Heterandria formosa* (GROVE & WOURMS 1991, 1994).

Independent of the degree of the transfer of maternal nutritive molecules, already light microscopists emphasized increase of vascularization of the follicle during gestation (see reviews cited above). At the ultrastructural level "junctional complexes" have been described between adjacent follicle cells one week after fertilization (and later) either without any further specification (JOLLIE & JOLLIE 1964b) or more precisely as junctional complexes, which include large tight junctions (zonulae occludentes). It was assumed that tight junctions develop after fertilization to reduce or prevent free diffusion of molecules across the epithelium, thus controlling epithelial exchange and probably immunological protection (GROVE & WOURMS 1994). However, the TEM-pictures published by JOLLIE & JOLLIE (1964 a, b) do not allow an unambiguous interpretation due to the insufficient fixation and findings reported in these early TEM-studies generally need re-examination, whereas magnifications of the pictures showing an early vitellogenic oocyte (GROVE & WOURMS 1994) are too low to demonstrate unequivocally the absence of (small) tight junctions. Authors did not use electron dense markers, e.g. lanthanum, often employed to visualize barriers created by tight junctions, such as the blood testis barrier (e.g. BERGMANN et al. 1984).

In the present note we used freeze fracturing, a technique widely used to visualize cell-cell contacts more accurately (e.g. STAEHELIN 1974, SHIVERS & MCVICAR 1995), but meanwhile a little out of fashion.

The ovary of anaesthetized nulliparous females were excised and fixed in 0.1 mol/l cacodylate buffer, pH 7.2, for several hours and then postfixed in 1% osmium tetroxide in the same buffer. Specimens were embedded in Epon, sectioned and stained with lead citrate. For freeze fracturing small pieces of the ovaries were fixed in 3% glutaraldehyde in 0.2 mol/l sodium veronal acetate, pH 7.3 for several hours at 4 °C. Specimens were infiltrated in 10%, 20% and 30% glycerol in the same buffer, frozen in liquid Freon 22 at -150 °C in a Balzer's freeze-etch unit BAE 301 and shadowed by platinum-carbon (45°) and replicated by carbon (90°). Replicas were cleaned in 40% chromic acid. Ultrathin sections and replicas were examined in a Philips EM 301.

Figure 1 a shows a vitellogenic oocyte and their surrounding follicle epithelium after conventional transmission electron microscopy, and figure 1 b a similar stage after freeze-fracture. Development of the zona pellucida has not finished as seen by the long microvilli, mainly of the oocyte penetrating this zone (fig. 1 a). Oocytes and follicle cells are easily to be distinguished (see also RIEHL & GREVEN 1991, GROVE & WOURMS 1991, 1994): the oocyte is characterized by yolk platelets, cortical alveoli, dictyosomes and a large nucleus with abundant nuclear pores. Follicle cells display many profiles of rough endoplasmic reticulum. Freeze-fracture reveals small junctional complexes comprising at least two distinct intercellular junction types. There are very small tight junctions with the typical belt-like structure, which is made up of a network of strands or fibrils on the P-face not excessively branched and grooves on the corresponding E-face. In addition, very small gap junctions, (nexus of approximately 55-70 µm in diameter), obviously not associated with the fibrillar network of tight junctions, are seen (fig. 1 e). In addition, some aggregates of particles have been found in the membrane of the microvilli of the oocyte (fig. 1 f).

Our findings unequivocally demonstrate the presence of very small and structurally simple tight junctions and gap junctions between the follicle cells surrounding vitellogenic oocytes of *H. formosa*, which still had a zona pellucida perforated by microvilli. Mature oocytes of this species possess a more homogenous zona exhibiting only a few radial canals, if any (GRAVEMEIER & GREVEN 2006). Thus, the development and presence of junctional complexes, primarily of tight junction, are not "post-fertilization specializations for intrafollicular gestation in *H. formosa* and other poeciliids" as speculated by GROVE & WOURMS (1994: 180). Development of tight junctions takes place much earlier forming a "blood-follicle barrier" (see TOSHIMORI & YASUZUMI 1979). The same holds for the formation of gap junctions as transverse routes of small molecules, e.g. ions, second messengers etc., to synchronize epithelial activities (e.g. PERACCHIA 1977). Aggregations of membranous particles in the microvillous membrane of oocyte microvilli remain unexplained; they may represent intramembraneous proteins such as ATPases, previously demonstrated in oocytes of *H. formosa* by cytochemical methods (RIEHL 1980), or gap junctions, as particle size is similar (5.5-6 nm). Gap junctions between the microvilli of the oocyte and the follicle cell have been described for some teleosts (e.g. KESSEL et al. 1985).

During gestation the efficiency of the barrier and communication between follicle cells may be improved. In tight junctions the "leakiness"

Fig. 1 a-f: Organization of vitellogenic oocytes of *Heterandria formosa* after conventional transmission electron microscopy (a) and freeze-fracture (b-e). **a** Note the developing zona pellucida (asterisks) and microvilli (arrows) of the oocyte; **b** an oocyte in a similar stage as in figure a; **c**, **d** typical strands of a tight junction between follicle cells; **e** gap junction (arrows) between follicle cells; inset: detail; **f** particle aggregations (arrowheads) of the microvilli membrane in an oocyte; fe follicle cell, oc oocyte.

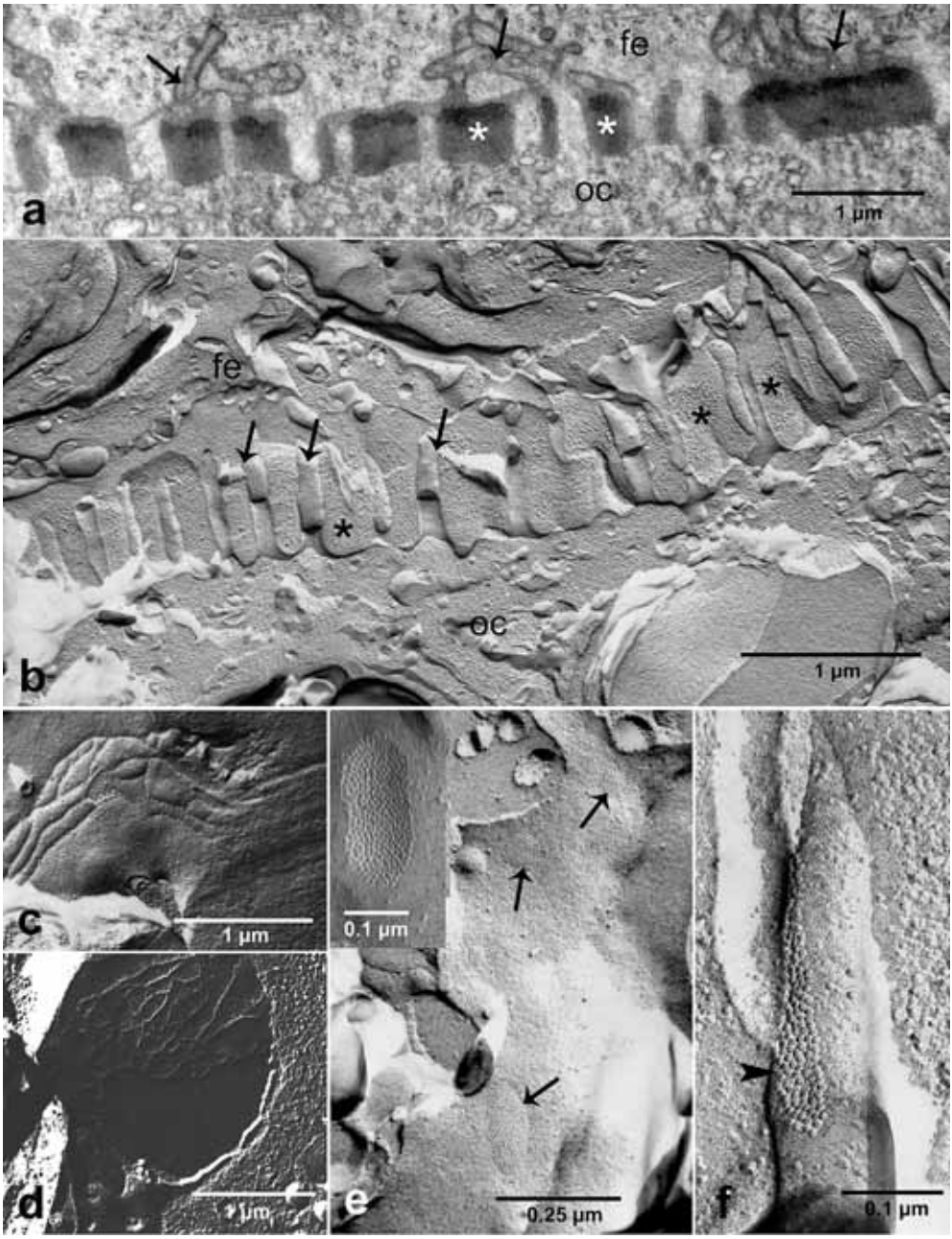

Abb. 1 a-f: Organisation einer vitellogenetischen Oocyte von *Heterandria formosa* nach konventioneller Transmissionselektronenmikroskopie (a) und Gefrierbruch (b-e). **a** Man beachte die sich entwickelnde zona pellucida (Sterne) und die Mikrovilli (Pfeile) der Oocyte (Pfeilköpfe); **b** eine Oocyte in einem vergleichbaren Stadium wie in Abbildung a; **c, d** typische Leisten einer zonula occludens zwischen Follikelzellen; **e** nexus (Pfeile) zwischen Follikelzellen; Einsatz: Detail; **f** Partikelaggreationen (Pfeilköpfe) in der Membran der Mikrovilli einer oocyte; fc Follikelepithel, oc Oocyte.

may be reduced by increasing their depth, which is indicated by the TEM-pictures of junctional complexes between follicle cells investing older embryos (GROVE & WOURMS 1994), and/or by increasing the number of strands; and gap junctions may increase their area (e.g. STAEHELIN 1974, PERACCHIA 1977).

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