Appendages of the oocyte nuclei in the gall midge *Heteropeza pygmaea*

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Summary. Spherical appendages of the nuclei in oocytes of the gall midge *Heteropeza pygmaea* were observed in ovaries cultured in vitro.

Time-lapse films of ovaries cultured in vitro of the paedogenetic gall midge *Heteropeza pygmaea* (Cecidomyiidae, Diptera) have revealed continuous pulsating movements of oocyte nuclei concomitant with nuclear constrictions during the entire period of follicle formation[2]. In a first note on these pulsating oocyte nuclei[3], the occasional occurrence of appendages of the oocyte nuclei was mentioned. We suggested a possible connexion between the pulsating movements of the oocyte nucleus and the origin of the appendages, as well as a possible identity of the nuclear appendages with the so-called 'small nuclei'. Small nuclei are found in addition to the oocyte nucleus in male-determined eggs but not in female-determined eggs during paedogenetic reproduction of *Heteropeza;* they contain 10 chromosomes[4-6]. In this report we give data on appearance and size of nuclear appendages in *Heteropeza* oocytes. We have also tried to obtain evidence for these suggestions as to the origin and fate of the appendages.

Material and methods. The methods used for in vitro culturing of ovaries and of the follicles they produce[7,8], and for time-lapse cinemicrography and electron microscope studies[2], have been described elsewhere. All ovaries were explanted from young larvae at the time of hatching from their mother larvae. The film sequences analyzed were the same as those used for the study of pulsating oocyte nuclei[2] and rotating follicles[9]. Follicles and mature eggs produced by the ovaries in vitro were fixed and stained according to the methods given by Camenzind[5].

Results and discussion. 13 cases of small spherical appendages of the oocyte nuclei were observed and followed in our films of developing ovaries (figure 1). These appendages usually appeared between 15 and 25 h after the start of the ovary cultures. The earliest observation of such an appendage was 7.5 h after explantation of the ovary. 1 oocyte nucleus was seen to have 2 such appendages. All nuclear appendages were attached to the oocyte nuclei, even during the pulsating movements of the oocyte nuclei. Similar to oocyte and nurse nuclei, the appendages increased in size gradually (figure 1; table). The diameter of 1 appendage was seen to increase in size from 2.1 to 4.7 µm in 11 h. It is conceivable that in some cases material from the oocyte nucleus slowly flowed into the appendage. This would imply the presence of a bridge between oocyte nucleus and appendage. Such a connexion between oocyte nucleus and appendage is shown in an electron micrograph in figure 2. However, in this case we do not know whether

Increase in size of oocyte nuclei, nuclear appendages of the oocyte nuclei and nurse nuclei during follicle formation

Mean value of diameter (µm)[a]	Time after the start of ovary cultures (h) 0–12	12–26	26–43
Oocyte nucleus	9.6±0.17	10.1±0.33	11.1±0.23
Nuclear appendage	2.3[b]±0.25	3.6±0.37	4.8±0.45
Nurse nucleus[c]	9.7±0.26	11.9±0.37	-[d]

[a] With 1 exception ([b]), the number of measurements ranged between 5 and 21. [b] Only 2 measurements. [c] Values refer to the large, germ-cell-derived nurse nucleus only (= g-trophocyte, Madhavan[10]). [d] No measurements.

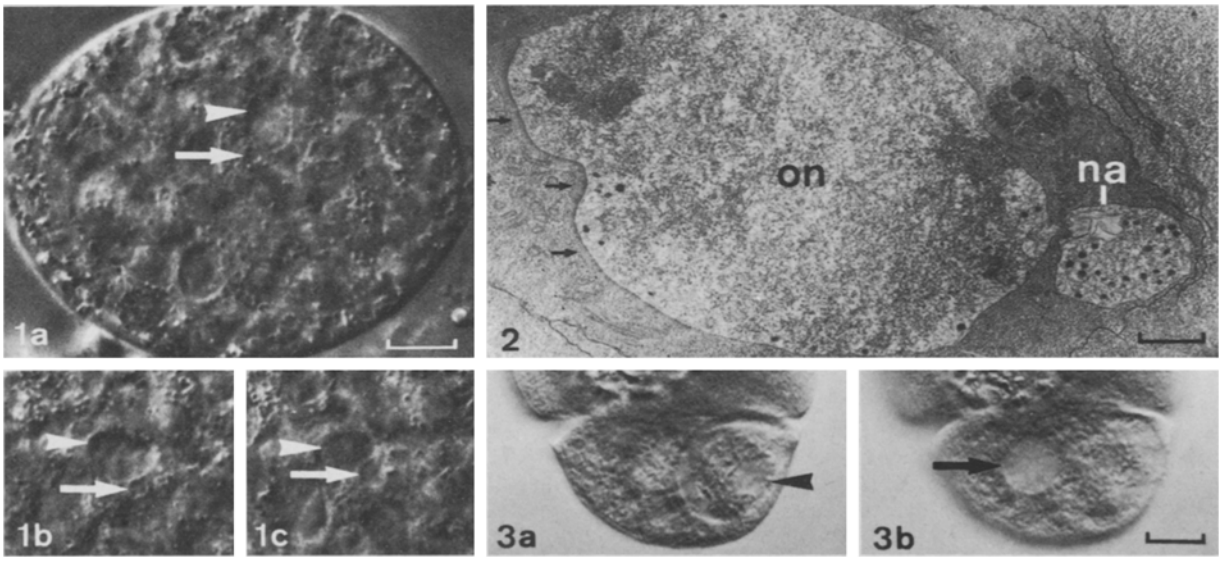

Fig. 1. 3 photographs taken from a 16-mm time-lapse film showing development of the *Heteropeza* ovary in vitro. Inside the ovary, a pulsating oocyte nucleus (arrowhead) with appendage (arrow) can be seen. The appendage slowly increases in size. The oocyte nucleus turns slightly during pulsation. It also increases in size (its greatest diameter is not in the focal plane in *c*). Time after explantation of the ovary is 8 h in *a*, 9 h in *b* and 16 h in *c* (bar = 10 µm, same magnification in all figures). Fig. 2. Ultra-thin-section of ovary showing oocyte nucleus (on) connected to nuclear appendage (na). The arrows point to a layer of filamentous material which covers part of the nuclear envelope of the oocyte. This layer is believed to be responsible for nuclear pulsation[2] (bar = 1 µm). Fig. 3. 2 photographs showing oocyte and part of the nurse chamber (on top) of immature follicle in different focal planes. Chromatin is seen in the oocyte nucleus (arrowhead) but not in the nuclear-like vesicle (arrow) (bar = 10 µm).

the appendage was stationary or whether nucleoplasm of the oocyte nucleus was pushed through the constriction into the nuclear bud in a pulsating movement. In none of the 13 cases in which the occurrence of nuclear appendages was filmed, could the origin of the appendages be ascertained. The site of the appendages suggested that they were formed by budding from the oocyte nuclei; in any case, involvement of pulsation of oocyte nuclei in the formation of the nuclear appendages could not be confirmed.

Some of these appendages were found in oocytes of ovaries which showed rapid development, i.e. which in all probability produced only female-determined eggs[11]. We were not able to follow these appendages in the films during oocyte growth, for accumulation of yolk granules obstructed the view. However, small nuclear-like vesicles were found in fixed material of immature and mature female-determined eggs produced in ovary cultures in vitro of a different series (figure 3). These nuclear-like vesicles were in most cases not (no longer?) attached to the oocyte nuclei. Chromosomal material was not found in them.

The observation that nuclear appendages were found in oocytes which most probably developed to female-determined eggs makes it unlikely that the nuclear appendages were transformed into small nuclei. The latter do not appear in female-determined eggs. Besides, it would be difficult to explain the occurrence of 10 chromosomes in the small nuclei. It seems more plausible to assume that the nuclear appendages were identical with the nuclear-like vesicles found in later stages of oocyte development.

The nuclear appendages and nuclear-like vesicles can be compared to the so-called 'accessory nuclei' which may occur in great numbers in oocytes of Hymenoptera and other insects[12,13]. These accessory nuclei probably arise by budding from the oocyte nucleus. Their function is still controversial. As yet we have found no indication of a possible function of the nuclear appendages in development of *Heteropeza pygmaea* oocytes.

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Secretory cell in the medulla of the bursa of Fabricius

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Summary. Light microscopy has revealed a possible secretory cell in the medulla of the bursa of Fabricius. Cyclophosphamide increased the presence of the secretory cell.

Research with the bursa of Fabricius has contributed significantly to our understanding of immunoglobulin synthesis in vertebrates[2]. We describe here the presence of a possible secretory cell in the medulla of the bursa and its cytological changes subsequent to treatment with cyclophosphamide.

Materials and methods. In our morphological studies, newly hatched 4-, 14-, 35-, 50-, and 120-day-old chickens were used. The bursal tissue samples were fixed in 4% buffered (0.2 M phosphate buffer) glutaraldehyde (2 h), rinsed 30 min in 0.2 M phosphate buffer (3 times), post-fixed in 2% osmium tetroxide (1–1.5 h), rinsed in buffer, dehydrated in ethanol, carried through propylene oxide, and embedded in araldite (Durcupan).

Results and discussion. Secretory cells were observed in all ages studied. They were localized in the medullary area and were usually parallel to the cortico-medullary border. We have found the largest number of cells in those follicles which were sectioned close to and parallel to the cortico-medullary border. In general, the secretory cell is elongated in shape, and the nucleus possesses a similar chromatin pattern to the small lymphocyte. The cytoplasm is dark and has 1 or 2 thick, long processes which contain dark granules of less than 1 μm in diameter (figure 1).

The secretory function of these cells became evident after cyclophosphamide (Cy) treatment. 7-week-old chickens (900–1100 g b.wt) were injected i.p. with Cy on 5 consecutive days. On the 1st day they were injected with 50 mg Cy/kg b.wt and on the following 4 days with 25 mg Cy/kg b.wt. The animals were killed 4 days after the last injection. The bursal tissues were prepared for histological investigation.

The lymphoid cells were eliminated from the follicles of Cy-treated birds. Histological structure of the follicles was the same as described by others[3–8]. In 1-μm sections stained with toluidine blue the bursa medulla of Cy-treated birds revealed a large amount of an intercellular dark substance (figure 2). The follicles of Cy-treated birds contained only epithelial cells, secretory cells, small lymphocyte-like cells, and plasma cells. Secretory cells were second in abundance to epithelial cells in the follicles of Cy-treated birds. The changes in secretory cell structure subsequent to Cy-treatment included modifications of the nucleus and the cytoplasm. The nucleus became round and revealed a leptochromatic pattern instead of the rough heterochromatic one of the normal animal. The cytoplasm was abundant and pale with fewer granules than in normal animals. Instead of long thick processes the cytoplasm was bulky, and exhibited spike-like processes embedded into the intercellular substance (figure 3). This cell type may be the same as mentioned by Glick[3] as large pale reticular cells or by Hoffman-Fezer et al.[8] as solitary thioninophilic cells. The intercellular substance appears initially around the cells containing granules (figure 4). When the light micrographs showed large amounts of an intercellular substance the