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Intermediate Nurse Cells in *Campodea spp.* (Diplura). Differentiation and Possible Role during Oogenesis*

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Synopsis

During previtellogenesis in *Campodea spp.* the nurse cells differentiate into two categories. Two cells adjoining the oocyte and connected with it, via intercellular bridges, develop into "intermediate cells" whereas other cells become typical nurse cells. The intermediate cells are morphologically and functionally transitional between the oocyte and nurse cells. The occurrence of such transitional cells confirms, as previously suggested, a gradient character of determinant controlling differentiation of the oocyte and nurse cells from the uniform group of sibling cystocytes.

Introduction

Previous studies have shown that the ovaries of Campodeidae are of the polytrophic meroistic type (Bareth, 1972; Asaba and Ando, 1978; Biliński, 1979). In *Campodea spp.* (Biliński, 1979) and *Lepidocampa* (Asaba and Ando, 1978) each egg chamber is composed of an oocyte and 20−30 nurse cells, which is an exception to the $N = 2^n$ rule (for review see Telfer, 1975). During previtellogenesis in *Campodea spp.* the nurse cells undergo additional differentiation: two cells connected with the oocyte, via intercellular bridges, develop into intermediate cells while other cells become typical nurse cells (Biliński, 1983b).

The aim of the present study was to describe the differentiation and development of the intermediate cells in the course of oogenesis. The results are discussed with reference to the possible function of these cells.

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Material and Methods

Specimens of *Campodea spp.* were collected between May and October in the Bieszczady National Park. After dissection, ovaries were fixed in 2% OsO_4 in 0.1 M phosphate buffer (pH 7.3). Sucrose was added to the fixative to produce a final osmolarity of 300 mosmol. After dehydration the material was embedded in Epon 812. Ultrathin sections were contrasted with uranyl acetate and lead citrate and examined in a Tesla BS 500 electron microscope at 60 kV. For routine microscopy, semithin epoxy sections were stained with 1% methylene blue in 1% borax. For the demonstration of RNA semithin sections, after removal of Epon (Maxwell, 1978), were stained with toluidine blue at pH 5 (Stockert, 1975).

Results

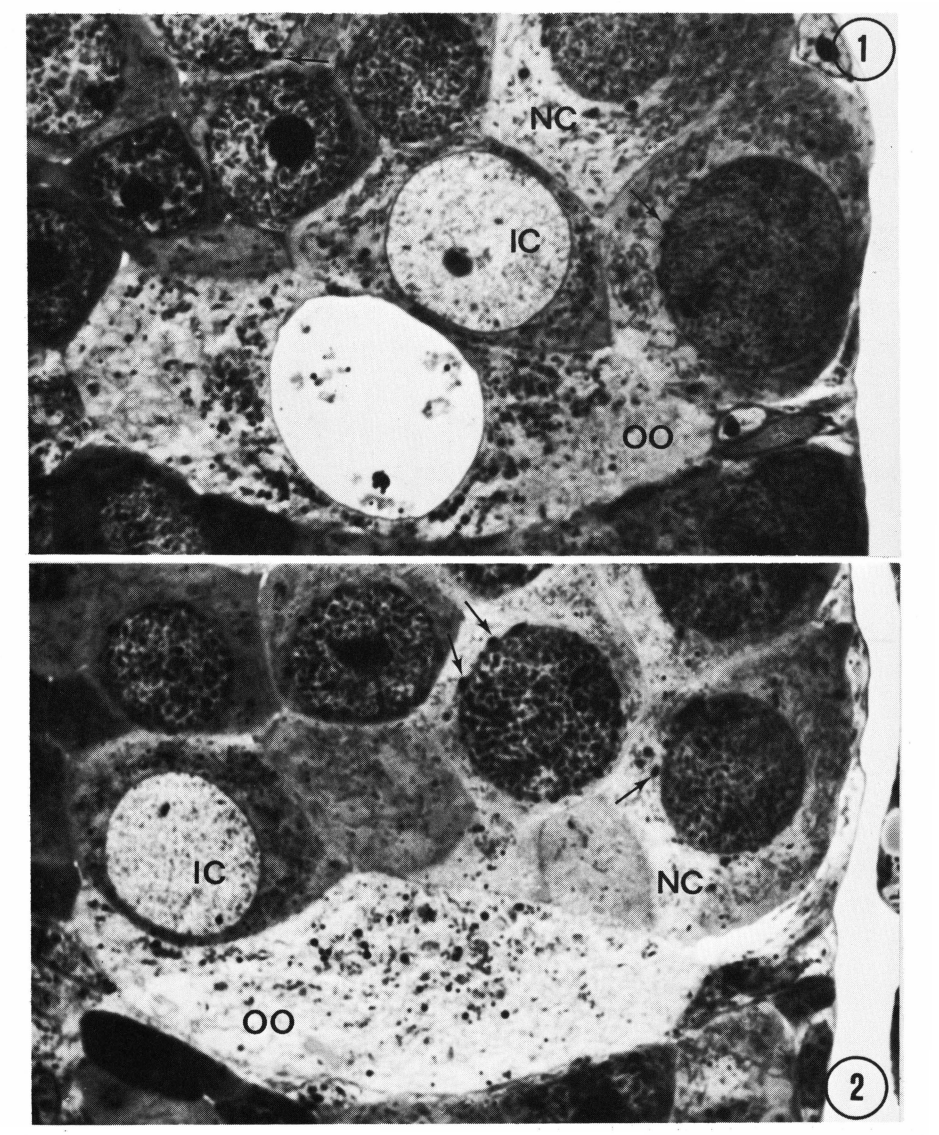
Previtellogenesis

The oocyte at this stage is a flattened cell *ca.* 40 μm in diameter. Its large nucleus is centrally located and translucent in light microscopic preparations (Fig. 1). Elements of rough endoplasmic reticulum (RER), mitochondria and numerous dense granules are present in the ooplasm. These latest structures occur predominantly near the nucleus (Fig. 1). The apical region of the egg chamber is occupied by the typical nurse cells (Figs. 1, 2). In the cytoplasm of these cells mitochondria, free ribosomes and accumulations of nuage material are observed (Figs. 1, 2, arrows). Spherical nuclei of the nurse cells contain densely stained nucleoli and numerous chromatin aggregations (Figs. 1, 2). In the central part of the egg chamber, between the oocyte and nurse cells, two "intermediate cells" occur. These cells most frequently adhere to the central part of the oocyte containing the nucleus (Fig. 1). The intermediate cells are, in some respects, transitional between the oocyte and nurse cells. In light microscopic preparations their nuclei, while "lighter" than the nuclei of typical nurse cells, are substantially "darker" than that of the oocyte (Figs. 1, 2). The accumulations of the nuage material, typical of nurse cells, are not observed in the intermediate cells.

Staining with toluidine blue at pH 5 for RNA demonstration gives following results: the nucleoli and the accumulations of nuage material are positive (Fig. 3) while the difference in the karyoplasm density between the intermediate and nurse cells is less conspicuous than in routine staining (compare Figs. 1—3).

Vitellogenesis

The intermediate cells at this stage of oogenesis still differ markedly from the nurse cells (Fig. 4). In the cytoplasm of the intermediate cell mitochondria, elements of RER, lipid droplets and numerous dense granules are observed (Figs. 4, 5). These latest structures are enclosed in dilated vesicles of RER (Fig. 5, arrows). It has been reported previously (Biliński, 1979), that identical granules are formed also in the oocyte cytoplasm (there termed type I yolk spheres). The ovoid nucleus of intermediate



Figs. 1, 2. Semiserial sections of an egg chamber during previtellogenesis. Note oocyte (oo), intermediate cells (ic) and nurse cells (nc). Arrows indicate accumulations of nuage material in nurse cell cytoplasm. Methylene blue. × 1,300.

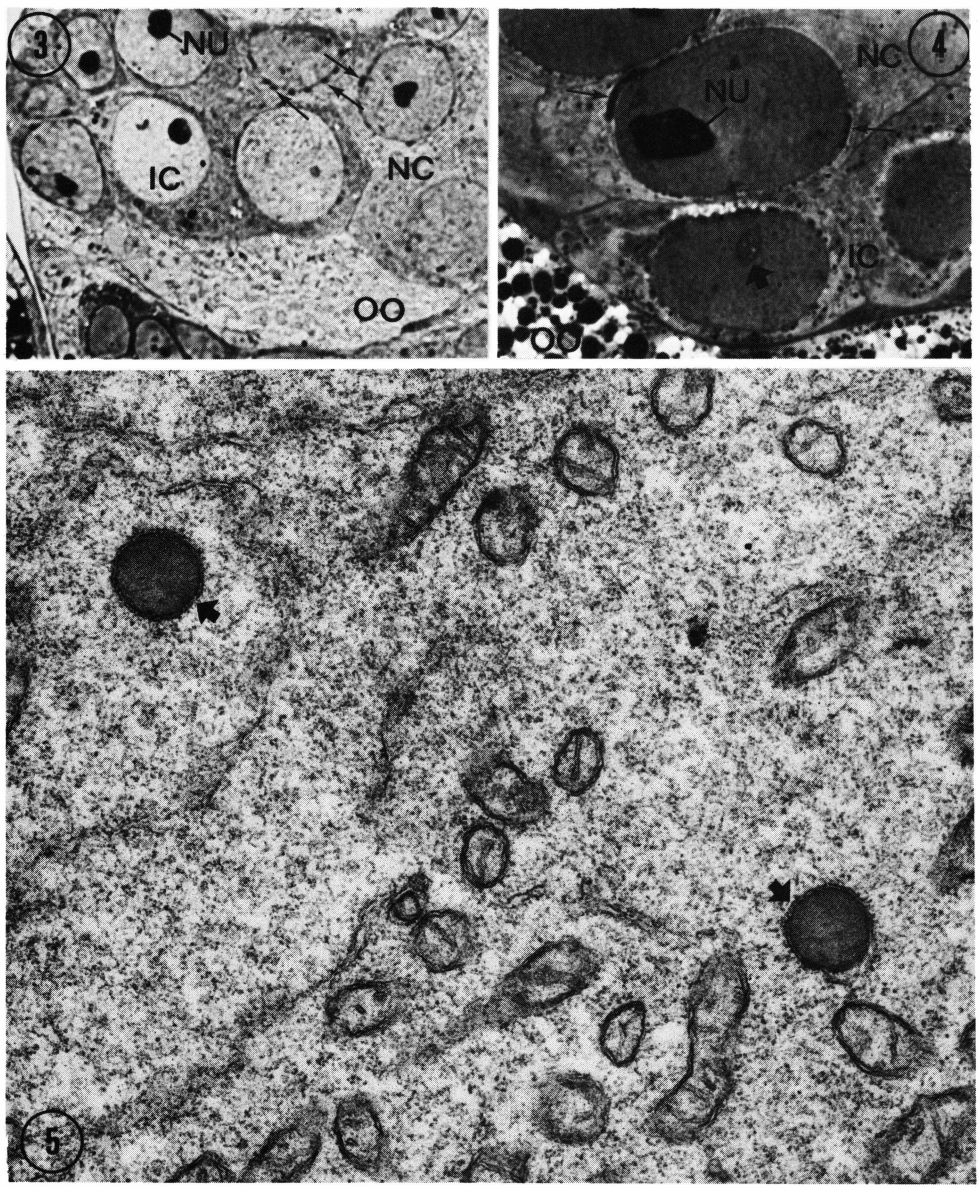


Fig. 3. Photomicrograph of an egg chamber during previtellogenesis. Note nucleoli (nu) and aggregations of nuage material (arrows). ic, intermediate cell; nc, nurse cells; oo, oocyte. Toluidine blue at pH 5. × 900.

Fig. 4. Photomicrograph of an egg chamber during vitellogenesis. Note dense granules and transparent lipid droplets in the cytoplasm of intermediate cells (ic). Arrow indicates weakly stained structure in the nucleus of intermediate cell. nc, nurse cell; nu, nucleolus; oo, oocyte; small arrows, aggregations of nuage material. Methylene blue. × 1,000.

Fig. 5. Portion of an intermediate cell. Note electron dense granules (arrows), mitochondria, RER elements and ribosomes. × 24,000.

cell contains only a single weakly stained structure (Fig. 4, arrow), that might be interpreted as residual, nonfunctional nucleolus.

Typical nurse cells are markedly larger than intermediate cells (Fig. 4). The nurse cell cytoplasm contains numerous mitochondria and RER elements, while dense granules, typical for the intermediate cells, are unfrequent. Elongated accumulations of nuage material are observed in close contact with nuclear envelope (Fig. 4, small arrows). Within the nucleus, large densely stained nucleolus occur (Fig. 4).

During vitellogenesis the ribosomes, lipid droplets and dense granules, produced by the nurse and/or intermediate cells, are transported to the oocyte. At the end of this stage both the nurse and intermediate cells degenerate.

Postvitellogenesis

During this developmental stage a thin granular chorion is deposited on the oocyte surface (Biliński, 1983a). Concurrently, segregation of reserve material takes place within the ooplasm. Dense granules migrate to peripheral ooplasm and make up a distinct cortical layer (Figs. 6, 7). Larger spheres, originating from pinocytotic vesicles, remain in the oocyte centre (Fig. 6).

Discussion

Previous studies have shown that the principal function of typical nurse cells in *Campodea* is to synthesize rRNA, that is later transported to the oocyte (Biliński, 1979). This sequence agrees well with generally accepted view of the role of trophocytes in oogenesis (reviewed by Telfer, 1975). It seems likely that the participation of the intermediate cells in rRNA synthesis is limited, as may be judged by the disappearance of their nucleoli. On the other hand, the intermediate cells show some features in common with the oocyte, *i. e.* they are able to synthesize dense granules and lipid droplets.

A comparison of results obtained by the routine and toluidine blue stainings show that the difference in the karyoplasm density between the nurse and intermediate cells results from different DNA rather than RNA content. This may suggest that these types of cells differ in the degree of endopolyploidy.

All these data show that the intermediate cells are transitional between oocytes and typical nurse cells not only morphologically but also functionally. The occurrence of such transitional cells confirms earlier reports (Biliński, 1983b) on gradient character of determinant responsible for differentiation of the oocyte and nurse cells from the originally uniform group of sibling cystocytes.

The egg chamber of *Campodea* shows much similarity to that of another apterygotan insect, *Tetrodontophora bielanensis*. In this species two nurse cells, connected directly with the oocyte, differ from remaining nurse cells (Krzysztofowicz, 1971, 1975). Autoradiographic and ultrastructural studies have shown that these two cells play no part in RNA synthesis(Krzysztofowicz, 1971, 1975). Among pterygotan insects all nurse cells, of a given chamber, are morphologically and functionally similar. It has been shown, however, that in some species nurse cell nuclei contain various amounts of

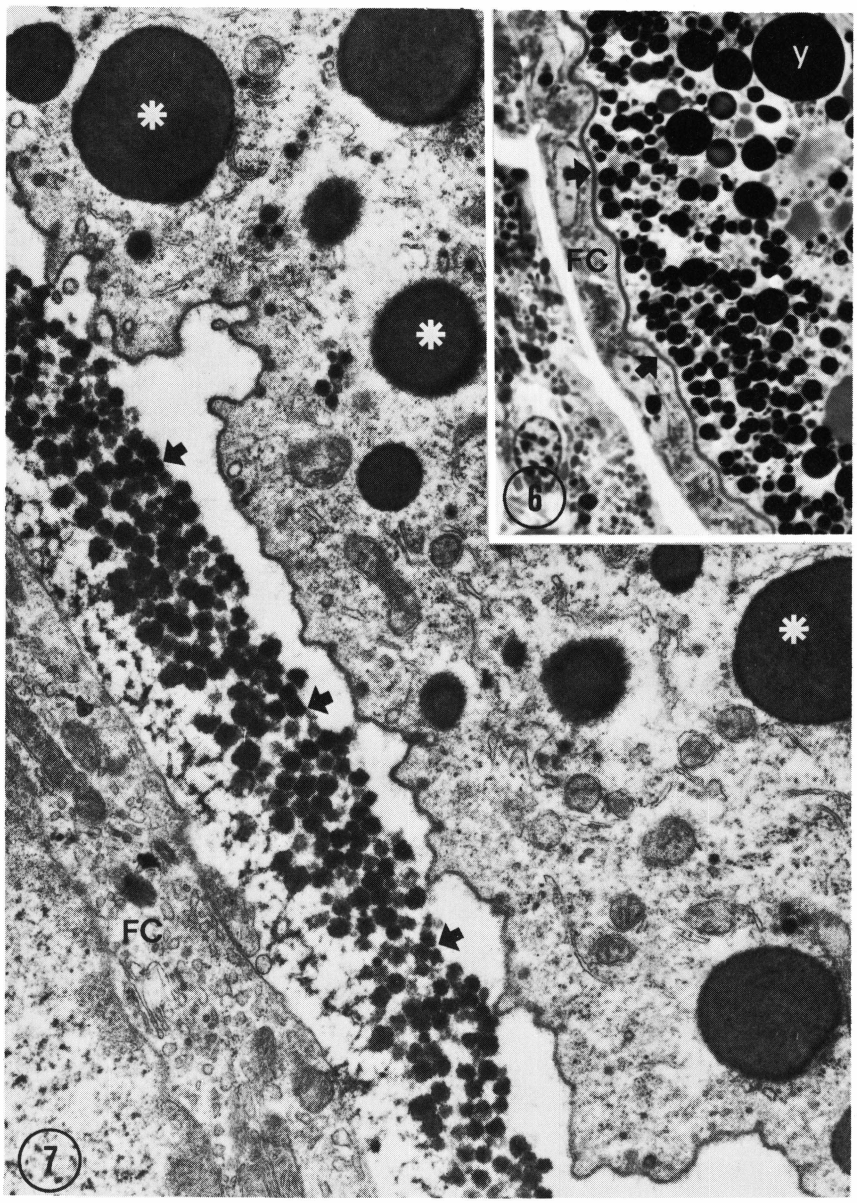


Fig. 6. Follicle cells (fc) during postvitellogenesis. Note chorion (arrows) and dense granules forming cortical layer of ooplasm. y, yolk spheres. Methylene blue. × 1,500.

Fig. 7. Cortical ooplasm filled with dense granules (asterisks). fc, follicle cell; arrows, chorion. × 19,000.

DNA, *i. e.* achieve various degree of endopolyploidy. In *Drosophila* the degree of ploidy in the nurse cells adjacent to the oocyte is 2 or 4 times greater than those of more distally located cells (reviewed by Telfer, 1975).

The dense granules, formed in the oocyte and intermediate cells have been previously described and called "type I yolk spheres" (Biliński, 1979). The mode of the formation of these granules, their migration to the oocyte periphery as well as their biochemical composition different from that of the yolk granules (Bareth, 1972; Asaba and Ando, 1978) show, however, that they represent yet another kind of reserve material. It might be suggested, that these granules contain the precursors of a substance which having been released from the oocyte, forms additional egg envelope or reinforces the chorion. This supposition is in line with the results of previous studies (Biliński, 1983a) showing that the chorion in *Campodea spp.* is thin and composed of loosely aggregated granules.

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