

ULTRASTRUCTURAL STUDY OF THE INTERCELLULAR BRIDGES BETWEEN FEMALE GERMINAL CELLS IN *CHIROCEPHALUS DIAPHANUS* PREVOST (CRUSTACEA: BRANCHIOPODA)

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RESUM

Estudi ultrastructural dels ponts intercel·lulars trobats entre les cèl·lules germinals femenines de *Chirocephalus diaphanus* Prevost (Crustacea: Branchiopoda)

S'ha efectuat un estudi en detall de la morfologia dels ponts intercel·lulars trobats entre les cèl·lules germinals femenines de ***Chirocephalus diaphanus***. Sembla que provenen de citodièresis incompletes durant el procés de multiplicació de les oogònies. En l'interior dels ponts es pot observar una gran varietat d'òrgànuls citoplasmàtics: mitocondris, reticle endoplasmàtic rugós, ribosomes lliures i vesícules que molt possiblement deriven dels dictiosomes, situats al voltant dels ponts. Cal destacar que en els ponts intercel·lulars que relacionen oòcits en estat de paquitè a la profase meiótica, el nombre de vesícules és més abundant.

En les zones properes als ponts intercel·lulars, l'espai intercel·lular presenta una dilatació en forma de canalicles, i es poden comptar un gran nombre d'unions cel·lulars del tipus **macula adherens**.

Pel que fa al paper dels ponts intercel·lulars, sembla que concorren a la creació d'un tipus de sincronisme de la diferenciació cel·lular en el decurs de la gametogènesi.

INTRODUCTION

The intercellular bridges which guarantee cytoplasmic continuity between two or more cells are, in general, considered to be the remains of incomplete cytokinesis and are not to be mistaken for the ephemeral intercellular communications which form during telophase. The latter are cons-

tituted by cytoplasmic constrictions whose central part is occupied by a dense disc of fibrillar material associated with remains of spindle microtubules known as the mid-body, intermediate-body, *zwischenkör* or Fleming body.

Although the existence of intercellular communications was known in classical cytology, ultrastructural studies have de-

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monstrated that the majority of supposed intercellular communications are nothing other than zones of intimate contact between neighbouring cells with no interruption in their plasmatic membranes; that is, tight junction.

During the first stages of gametogenesis one frequently finds intercellular bridges between the different types of cells constituting the gonads. In the female gonad the bridges may be presented:

a) Between germinal cells of the same category both in invertebrates: *Drosophila melanogaster* (KING & DEVINE, 1958; BROWN & KING, 1964), *Libellula* (KESSEL & BEAMS, 1969), *Artemia salina* (ANTEUNIS *et al.*, 1966), *Mytilicola intestinalis* (DURFORT *et al.*, 1980), as well as in vertebrates: rat (FRANCHI & MANDL, 1962), mouse (RUBY *et al.*, 1969), *Rana pipiens* (RUBY *et al.*, 1970), *Mesocricetus auratus* (SANTOS CARDO, 1974).

b) Between follicular or nurse cells: *Drosophila* (GIORGI, 1978), *Culex pipiens* (FIIL, 1978), *Apis mellifica* (RAMAMURTY & ENGELS, 1977).

c) Between germ cells and gonad nurse, follicular or accessory cells: *Diopatra cuprea* (ANDERSON & HUEBNER, 1968), *Drosophila melanogaster* (KING & DEVINE, 1958; BROWN & KING, 1964; KOCH & KING, 1969; CUMMINGS *et al.*, 1969), *Dytiscus marginalis* (STEINER & URBANI, 1969).

In the same way, intercellular bridges have been described between oocytes and follicular cells of teleostean fish, amphibians, reptiles, birds and mammals, and even in man (BACA & ZAMBONI, 1967).

With regard to the presence of intercellular bridges between male germ cells, we must point out that they have likewise been found in various groups, both in invertebrates as well as vertebrates. A wide range of references exists on the subject, but here we only mention some of the first papers which appeared, such as those of BURGOS & FAWCETT (1955), FAWCETT *et al.* (1959), ITO (1960) and KRISHAN & BUCK (1965). For further information on the subject we would cite the papers by BRUSLÉ (1971, 1972).

Intercellular communications have also been observed between somatic cells of different lineage: cnidoblasts of *Hydra* (FAWCETT, 1961), embryonic pancreas of rat (CADETE-LEITE, 1974), rat erythroblast (BUCK & TISDALE, 1962), HeLa cells (ERLANDSON & DE HARVEN, 1971), blastomeres of sea-ur-

chin eggs (WOLPERT & MERCER, 1961), fertilized egg of *Artemia salina* (FAUTREZ-FIRLEFYN & FAUTREZ, 1962).

With respect to the functional role of the cytoplasmic bridges, ZAMBONI & GONDOS (1968), on studying the evolution of the female germ line in mammals, suggest a preponderant participation of these bridges in the synchronised differentiation of the germ cells. This confirms FAWCETT'S (1961) theories relating to the intercellular bridges present in the germinal epithelium of the testicle, which play an essential role in the synchronisation of the development of the male gametes.

This paper studies the morphology of the intercellular bridges observed between female germ cells during the first stages of oogenesis of the branchiopod anostracan *Chirocephalus diaphanus*.

MATERIAL AND METHODS

The specimens of *Chirocephalus diaphanus* studied come from some ephemeral small lakes of peaty substratum, situated at a height of 172 metres above sea-level in the municipality of Capmany (Girona, Spain). They were collected during the months of March and April, 1983.

Once the gonads were isolated under stereomicroscope they were fixed with 2 % paraformaldehyde - 2.5 % glutaraldehyde mixture (KARNOVSKY, 1965) buffered with 0.2M Sörensen at pH 7.4 for one hour at 4°C. After careful washing with the said buffer, a postfixation for one hour at 4°C was performed with 2 % osmium tetroxide in the same buffer.

After alcoholic dehydration and using propylene oxide as an intermediate liquid, the samples were then embedded in Durcupan ACM (Fluka).

The thin sections, obtained with a Reichert Om-U2 ultramicrotome, were stained with uranyl acetate (WATSON, 1958) and lead citrate (REYNOLDS, 1963), and observed by means of the Philips EM-200 and EM-301 transmission electron microscopes from the Servei de Microscòpia Electrònica of the Universitat de Barcelona.

The thick sections of 0.5 and 1 µm in thickness, used as controls before obtaining the thin sections, were stained with a mixture of equal parts of 1 % azur II and 1 % methylene blue in 1 % borax (RICHARDSON *et al.*, 1960).

RESULTS

The evolution of the germ line in the ovary of *Chirocephalus diaphanus* takes place in germinal nests, whose cells are to be found in the same stage of differentiation. The ultrastructural observation of the germ cells which form the germinal nests—oogonial cells and oocytes I— shows the presence of intercellular bridges between them. The number and arrangement of these bridges suggests that practically all the cells of a germinal nest communicate with each other, giving the whole a syncytial character (Fig. 1).

The cytoplasmic communications observed between germ cells of the same category must be considered as open type intercellular bridges, on presenting the usual morphology of this type of structures. So, around the isthmus of cytoplasmic communication, the membranes of the two adjacent cells separate forming a more or less triangular widening in the intercellular space, to join again at a point of inflexion, giving rise to a club-shaped structure (Figs. 2, 5, 6). The plasmatic membrane which delimits the bridge, that is, the widest part of the club, presents a thickening

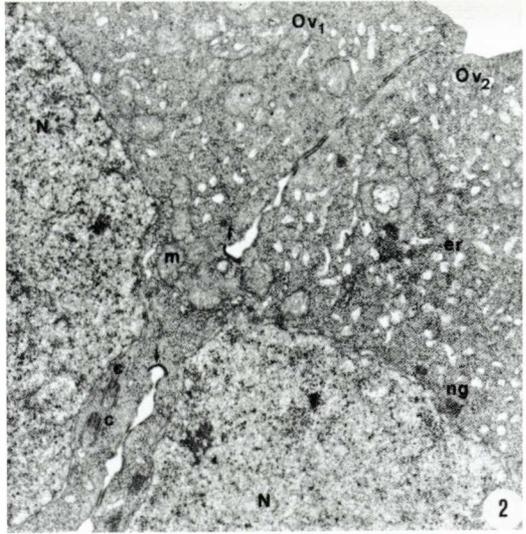


FIG. 2. Oocytes (Ov_1 , Ov_2) communicating by an intercellular bridge. In this perpendicular section, the bridge appears as two club-shaped structures facing one another and separated by the diameter of the bridge. (X 37.934).

Oòcits (Ov_1 , Ov_2) comunicats per un pont intercel·lular. En secció perpendicular, al pont s'observa com dues estructures amb forma de maça. (X 37.934).

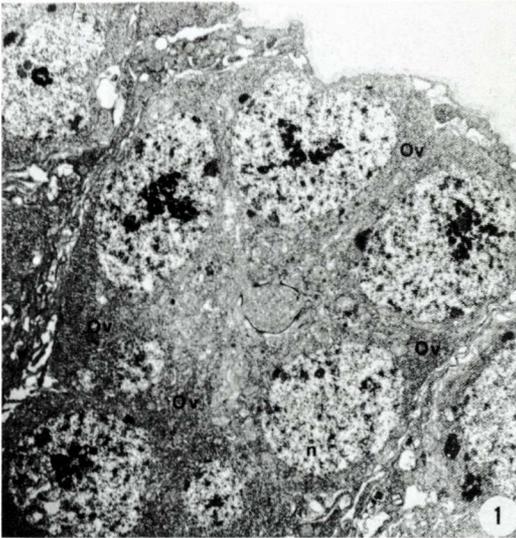


FIG. 1. Section of a germ nest formed by several oocytes. Between the cells intercellular bridges can be seen. (X 19.314).

Secció d'un cist format per diversos oòcits. Entre les cèl·lules s'observen els ponts intercel·lulars. (X 19.314).

of some 30 Å on its internal face, constituted by a certain amount of highly osmiophilic material arranged in transverse bands (Figs. 5, 6).

Study of the cross and tangential sections allows one to observe the intercellular bridges as circular holes approximately 1.5-2 μm in diameter, surrounded by a ring (Fig. 1, 3). In the perpendicular sections, two club-shaped structures are observed, one opposite the other and separated by the diameter of the bridge (Fig. 2).

Within and around the bridges, a great variety of cytoplasmic organelles can be observed: mitochondria, endoplasmic reticulum both smooth and rough, as well as numerous free ribosomes (Fig. 2).

It is interesting to point out the presence of numerous vesicles of different sizes within the intercellular bridges (Fig. 4). These vesicles are similar, with respect to their morphology, to those associated with Golgi's apparatus adjacent to the bridge, and it is highly probable that they have the same golgian origin (Fig. 4). The number of vesicles inside and near to the bridges increases considerably in the oocytes which are in the pachytene stage of meio-

tic prophase. During this phase, the nuclei of the oocytes are characterised by the presence of synaptinemal complexes.

Likewise, in the oocytes of *Chirocephalus diaphanus* a certain topographical relationship has been observed between the centriolar apparatus and the intercellular bridges.

Intercellular bridges have been observed between the germinal cells in the oogonia phase and the oocyte. I phase, this being more frequent between the oocytes in meiosis than between the gonial cells during multiplication.

Finally, around the intercellular bridges, one frequently finds areas in which the intercellular space presents considerable dilatations like intercellular canals, whilst at the same time cell contacts of the *macula adherens* type are generally observed (Figs. 5, 6, 7).

DISCUSSION

The description of the intercellular bridges which exist in the female gonad of *Chirocephalus diaphanus* coincides with the results obtained in other crustaceans: *Artemia salina* (ARTEUNIS *et al.*, 1966), *My-*

tilicola intestinalis (DURFORT *et al.*, 1980), although both in *Artemia* and *Mytilicola* two types of bridge exist: open and occluded.

This structural uniformity suggests that the intercellular bridges are formed by a unique process. The hypothesis most widely accepted is that which holds that, starting from the ultrastructural aspect of the bridges and their central situation in the plasmatic membranes of two communicating cells, the intercellular bridges are remains of incomplete cytokinesis (BURGOS & FAWCETT, 1955; FAWCETT *et al.*, 1959; FAWCETT, 1961). That is to say, during telophase the remains of the spindle end by disintegrating whilst a narrow isthmus of cytoplasm persists, establishing a communication between the daughter cells.

In the case of the ovary of *Chirocephalus diaphanus* this hypothesis can be assumed. The fact that it presents germ-nests, whose cells are to be found in the same stage of differentiation and the majority of which are joined by means of intercellular bridges, may be due to successive divisions, all incomplete, which provide the whole with a syncytial organisation.

The majority of germ-nests cells will be-

FIG. 3. Tangential section through the osmiophilic ring surrounding the intercellular bridge. (X 47.840).
Secció tangencial a nivell de l'anell osmiòfil que voreja el pont intercel·lular. (X 47.840).

FIG. 4. Detail of the portion of cytoplasm bordered by the intercellular bridges. (X 47.840).
Detall de la porció de citoplasma clos pel pont intercel·lular. (X 47.840).

FIGS. 5, 6. Detail of the widening of the intercellular space which gives rise to the club-shaped structure. Note the osmiophilic thickening of the internal face of the plasmatic membrane at the point of inflexion (arrows). (Fig. 5: X 89.600; Fig. 6: X 103.936).
Detalls de l'eixamplament de l'espai intercel·lular que dona lloc a l'estructura en forma de maça. S'observa un engrossiment osmiòfil a la cara interna de la membrana plasmàtica a nivell del punt d'inflexió (sagetes). (Fig. 5: X 89.600; Fig. 6: X 103.936).

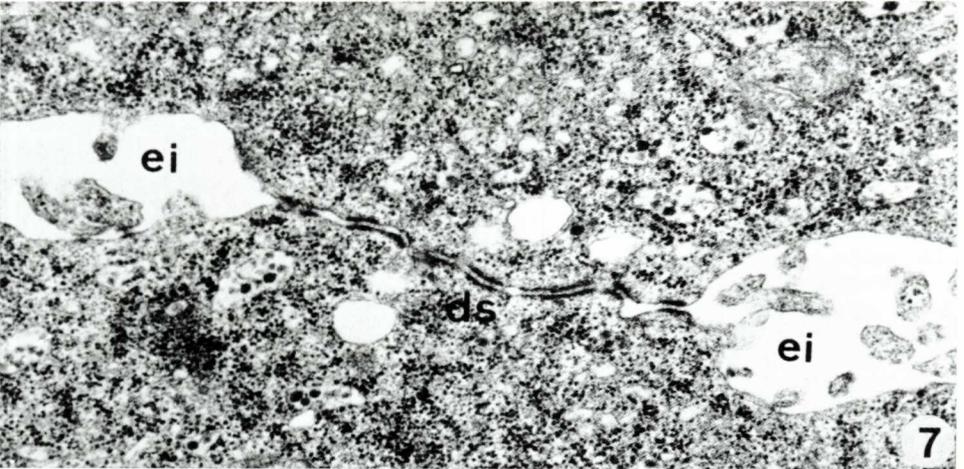
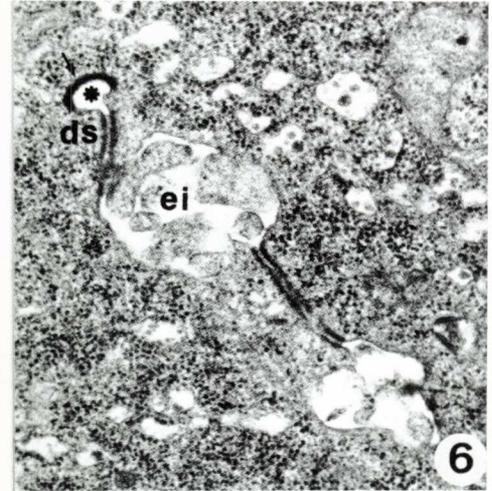
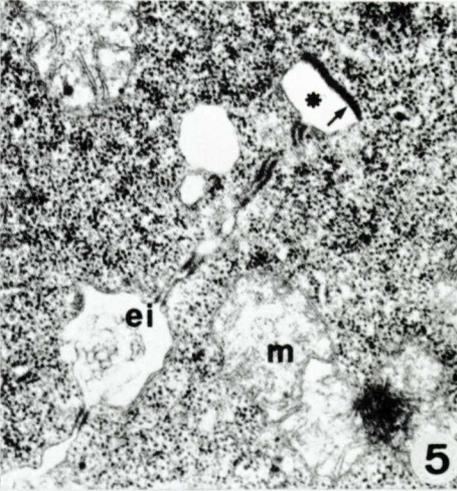
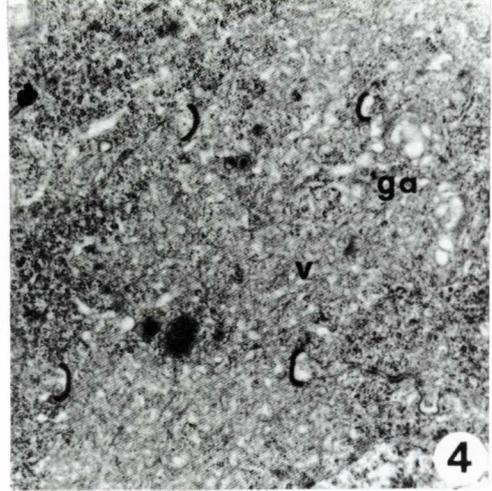
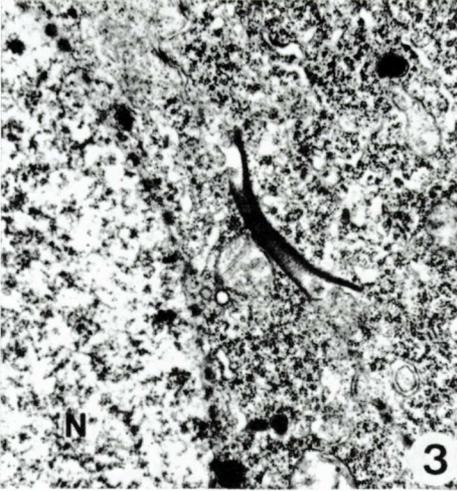
FIG. 7. Intercellular space showing dilatations in the form of intercellular canals and cell contacts of the *macula adherens* type. (X 97.760).
Espai intercel·lular que presenta dilatacions a manera de canalicles intercel·lulars i contactes cel·lulars del tipus *macula adherens*. (X 97.760).

Symbol key for the figures

c. Centriole
ei. Intercellular espace
er. Endoplasmic reticulum
ga. Golgi apparatus
m. Mitochondrion
ds. Desmosome (*Macula adherens*)
n. Nucleus
ng. Nucleolus emission (*nuage*)
Oc. Oocytes
v. Vesicles

Clau de símbols de les figures

Centriol
Espai intercel·lular
Reticle endoplasmàtic
Aparell de Golgi
Mitochondri
Desmosoma (*macula adherens*)
Nucli
Emissions nucleolals (*nuage*)
Oòcits
Vesícules



come nurse cells. Only a few cells will reach the phase of vitellogenesis and be converted into mature oocytes apt for fecundation. It is characteristic of the oogenesis of a large number of invertebrates to find cells of the same lineage which initially present a synchronised development but later have different potentiality, so that whilst some give rise to mature oocytes, others will be transformed into nurse cells. This occurs in the case of *Drosophila melanogaster* (BROWN & KING, 1964), *Dystiscus marginalis* (STEINER & URBANI, 1969), *Chironomus* (WULKER & WINTER, 1970), *Artemia salina* (ANTEUNIS *et al.*, 1966), and *Diopatra cuprea* (ANDERSON & HUEBNER, 1968), amongst others.

Regarding functional significance, the presence of cell organelles in the cytoplasmic area of the intercellular bridges suggests that these communications must serve as canals for transferring substances, organelles and genetic information between the germ cells related in this way. Certain authors believe that this transfer of organelles and materials from one oocyte to another may provoke a deficiency of such cytoplasmic constituents in some oocytes, causing a possible differentiation and even cell degeneration. In the oogenesis of mammals it has been observed that mitosis is followed by a degeneration of the majority of oocytes. Thus, GONDOS & ZAMBONI (1969) believe that the oocytes which do not lose their intercellular communications are the ones which are destined to degenerate.

In *Chirocephalus* vacuoles and vesicles have been observed within and around the intercellular bridges whose origin is probably to be found in the adjacent Golgi apparatus. The spectacular development that Golgi's apparatus, as well as the vesicles derived from it experience in the oocytes of *Chirocephalus diaphanus* during the pachytene stage of meiosis, is similar to that described by BAKER & FRANCHI (1967) in human oocytes and by RUBY *et al.* (1969) in rat oocytes. The morphology of the vesicles studied is similar to that of the vesicles described in *Ophioderma* (KESSEL, 1968), *Limulus* (DUMONT & ANDERSON, 1967), and in the mouse (RUBY *et al.*, 1969).

It would seem that the interchange of substances, organelles and genetic information between germ cells related by the intercellular bridges, contributes to the creation of a certain synchronism of cell

differentiation during the period of multiplication and at the beginning of meiotic prophase.

CONCLUSIONS

1. During the first stages of oogenesis of *Chirocephalus diaphanus*, the germinal cells are grouped in germ nests.

2. Amongst the germinal cells which form the germ nests, cytoplasmic communications are observed—intercellular bridges—which confer a syncytial character to the whole.

3. Given their morphology, the cytoplasmic communications observed in *Chirocephalus diaphanus* must be considered as intercellular bridges of an open type. They originate from incomplete cytokinesis during the multiplication of the oögonial cells.

4. Within the bridges, a great variety of cytoplasmic organelles are observed: mitochondria, rough endoplasmic reticulum, free ribosomes and even vacuoles or vesicles which appear to be derived from Golgi's apparatus situated in the cytoplasm adjacent to the intercellular bridge.

5. The presence of vesicles and organelles in the cytoplasm of the intercellular bridges suggests that these communications serve as canals to transfer organelles and genetic information between different oocytes. It seems that the interchange of diverse materials and information between germ cells by means of the bridges contributes to the creation of a certain synchronism of cell differentiation during the first stages of oogenesis.

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