The Origin of the Nurse Chamber in Ovaries of Miastor (Diptera: Cecidomyidae)*

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Summary. The nurse chamber in Miastor is a syncytium containing a variable number of nuclei. Many workers have claimed that all the nurse nuclei were derived from somatic cells. However, since one of these nuclei is larger than the remaining nuclei, other workers have thought that this nucleus was germinal in origin. Our ultrastructural studies of the nurse chamber-oocyte complex demonstrate the presence of an intercellular bridge between the nurse chamber and the oocyte. Furthermore, the large nurse nucleus contains lamellae characteristic only of the germ line. Both findings indicate that one of the nurse nuclei is the sister to the oocyte.

Introduction

Ovarian development in Miastor and related species is unique among dipterans in that the nurse chamber of these polytrophic ovaries is a syncytium (cf. review in Mahowald, 1972). Many investigators (Kahle, 1908; Hegner, 1914), the most recent being Panelius (1968) in his studies of Heteropeza pygmaea, have claimed that all of the progeny of the pole cells or primordial germ cells become oocytes, and that all of the nurse chamber nuclei derive from somatic cells. Other workers (Reitberger, 1941; Hauschteck, 1962; Camenzind, 1966; Counce, 1968) have noticed that one of the nuclei in the nurse chamber is exceptionally large and may be of germinal origin. Madhaven (1973) has restudied the cell lineages of the primordial germ cells in Heteropeza, and he concludes that the large nucleus is germinal in origin. Inasmuch as there is extensive loss of chromosomes in somatic cells in these organisms while only the germ line retains the complete complement (Kahle, 1908; Nicklas, 1959), nuclear size would appear to be a good criterion of germinal origin. Further evidence in support of Madhaven's conclusion have been obtained in this ultrastructural study of Miastor.

During an earlier study of Miastor (Mahowald, 1972, 1974), one intercellular bridge or ring canal was found between the nurse chamber and the oocyte. Since this structure results from an incomplete cytokinesis (Burgos and Fawcett, 1955; Fawcett et al., 1959; Meyer, 1961; Fawcett, 1972), it appeared that the nurse chamber must have been derived by division of the oogonium. In order to clarify the origin of the nurse chamber, we have studied with the electron microscope

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larval ovaries at the time the nurse chamber is forming. From these observations, we conclude that one nurse nucleus is a sister of the oocyte nucleus.

Material and Methods

The Miastor lines were obtained from Dr. S. J. Counce and were reared according to her culture methods (Counce, 1966 and personal communication; Mahowald, 1974). This strain reproduces paedomogenetically and parthenogenetically. Larvae of the proper stage were immersed in fixative (2% glutaraldehyde, buffered at pH 7.4 with 0.1 M phosphate) (Sabatini et al., 1963) and punctured with a tungsten needle. The larvae were then transferred to fresh fixative for 30 to 60 minutes, then washed in 0.2 M sucrose in 0.1 M phosphate buffer, post-fixed with osmium tetroxide, and embedded in Araldite (Luft, 1961). Successive 0.5 μm or 1 μm sections were cut of the larvae, stained with toluidine blue, and examined with the light microscope for the presence of the ovary in the section. When the ovary was found, thin sections were cut, mounted on carbon films, and stained with uranyl and lead salts (Frasca and Parks, 1965).

Observations

The nurse chamber in Miastor and related organisms is always a syncytium containing a variable number of nuclei (cf. Matuszewski, 1968, for review). One typical intercellular bridge is found between the syncytial nurse chamber and the oocyte (Fig. 1). The diameter of the bridge is only 1–2 μm and this small size probably explains why its presence has not been noticed in earlier light microscopic studies. In order to determine the origin of the bridge, we examined ovaries both at the time of formation of the oocyte-nurse chamber complex and also throughout oogenesis. One intercellular bridge was found between the syncytial nurse chamber and oocyte at every stage (Figs. 1, 2). The presence of only one bridge indicates that only one of the syncytial nurse nuclei is a sister to the oocyte. This corresponds to the observations previously mentioned that one nurse nucleus is larger due to the greater amount of chromatin in the germ line (Nicklas, 1959).

The ultrastructure of the intercellular bridge is characteristic of dipterans. The two plasma membranes are usually closely opposed (Fig. 3). On the surface of the bridge an electron dense deposit is found (Figs. 3, 4) which is approximately 15 nm thick. In tangential sections of the dense edge, the deposit is present as periodic dense accretions, 10 to 15 nm in size and spaced every 30 nm (Fig. 4). In Miastor these dense accretions, both because of their extreme electron density and their smaller size, are probably not related to microtubules. Cassidy and King (1969) have described similar periodic dense accretions along the edges of the intercellular bridges in overies of Habrobracon which they have interpreted as due to microtubules. Abundant microtubules (Figs. 3, 4) are present in the intercellular bridge at this time, but are absent later.

The intercellular bridge has a convoluted surface at the time of its formation (Figs. 1–4). No evidence has been found that these folds are discontinuous so as to form leaflets as in Habrobracon (Cassidy and King, 1969) and Drosophila virilis (Kinderman and King, 1973). As the bridge becomes older, the edge becomes more evenly shaped as in Drosophila melanogaster (Meyer, 1961; King, 1970).

In the earliest stages examined with the electron microscope, both the oocyte and the large nurse nucleus contain nuclear lamellae (Fig. 2) (Mahowald, 1974;