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Structural relationships among central cell and egg apparatus cells of barley as related to transmission of male gametes

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Abstract

Barley embryo sacs were examined using light and electron microscopy before and during fertilization. One synergid degenerates after pollination with loss of nuclear and cytoplasmic organization and cell wall material between synergid and central cell. Some wall between egg and central cell is also lost. After pollen tube discharge into the degenerate synergid, the male gametes leave the synergid entering a pocket of central cell cytoplasm separated from the synergid only by membranes. This could provide for efficient gamete transmission and possible recognition through specific membrane contacts.

INTRODUCTION

Many studies since the early 1960's have indicated a role for one synergid in fertilization in flowering plants (Jensen, 1972), Cass and Jensen (1970) described fertilization events in barley including the demonstration of a sperm nucleus in one synergid. The synergid in which the sperm nucleus was identified undergoes degeneration after pollination but before pollen tube entrance into its cytoplasm.

This paper correlates information from both light and electron microscopy in an attempt to provide an understanding of modifications among the central cell and egg apparatus cells of barley which may lead to efficient male gamete transmission.

MATERIAL AND METHODS

Tissues for light microscopy were freeze-substituted, embedded in Epon 812, sectioned at 1.5 μm, and stained with periodic acid-Schiff's (PAS) and aniline blue black (Cass, Jensen, 1970). Identification of the sperm nucleus and certain embryo sac nuclei was done by the Feulgen method (Jensen, 1962) and/or by autoradiography using tri-

Figs 1, 2. Brightfield micrographs of barley egg apparatus cells before pollination. Fig. 1 is longitudinal section showing egg (E) and synergids (S). 900 ×. Fig. 2 is transverse section showing arrangement of egg and synergids. Cytoplasm immediately outside egg apparatus is that of central cell. 1000 ×

Figs 3, 4. Electron micrographs of egg apparatus cells after pollination. Fig. 3 shows darkened profile of degenerate synergid with embayment of central cell cytoplasm (CC) indicated by unlabeled arrows. Persistent synergid (PS) with its filiform apparatus (FA) to right. 4300 ×. Fig. 4. shows detail of embayment (CC) and presence of thickened wall outside (arrowheads) and lack of internal wall 10.300 ×

tiated actinomycin D (Cass, Jensen, 1970). Tissues for electron microscopy were fixed in glutaraldehyde and osmium tetroxide (Cass, Karas, 1975) with certain changes (Carroll, Mayhew, 1976), and were embedded in low viscosity resin (Spurr, 1969).

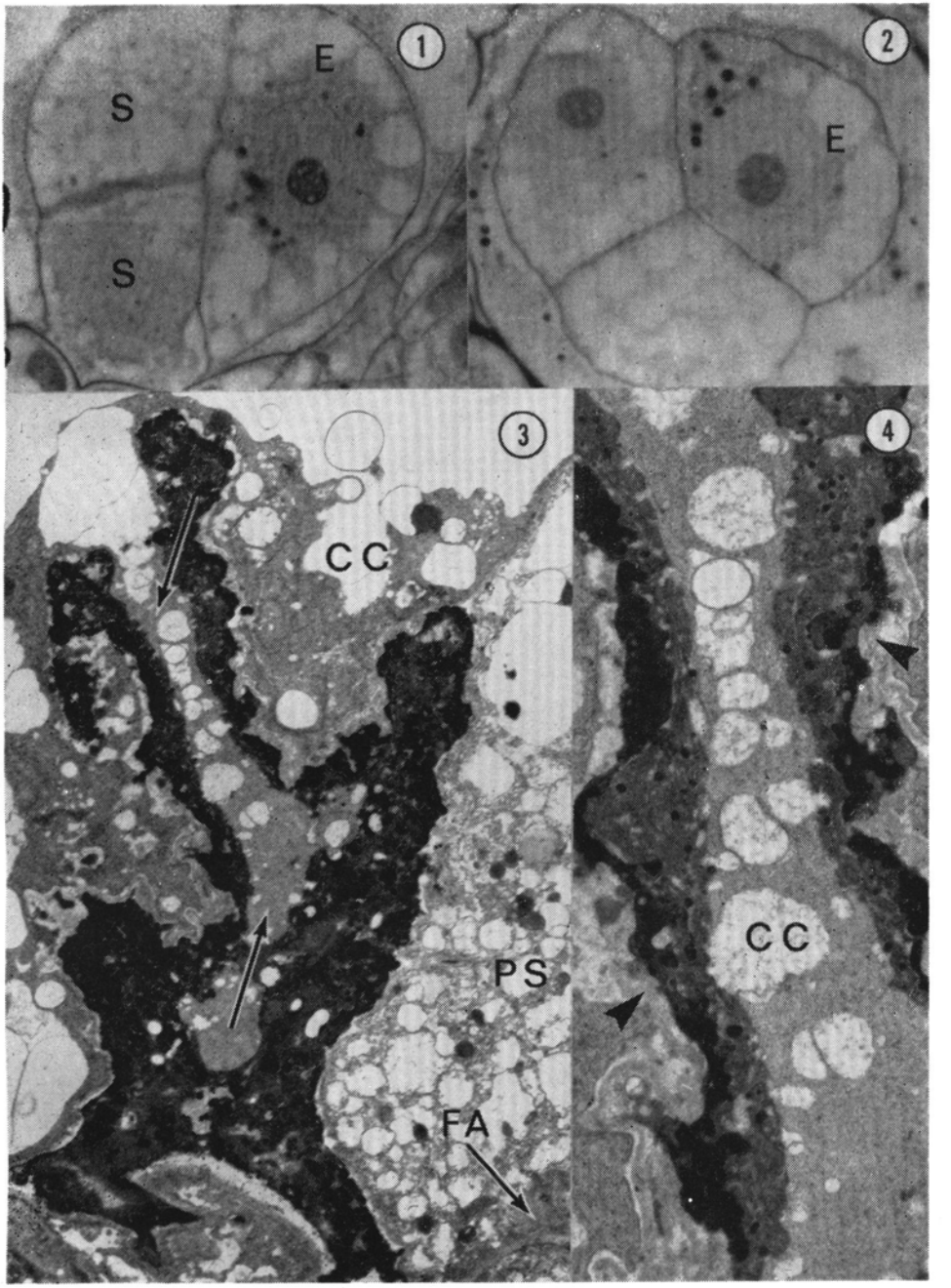
OBSERVATIONS

The egg apparatus of barley is 3-celled and is invested with central cell cytoplasm along its chalazal surface. Two partially fused polar nuclei occur in central cell cytoplasm along with abundant starch-containing plastids. Starch occurs in the egg but not in the synergids or antipodals. Organization of the egg apparatus cells prior to pollination can be seen in Figs 1, 2.

After pollination one synergid undergoes degeneration (Figs 3-7). Although each embryo sac cell in barley except the central cell possesses its own wall prior to pollination, specific loss of cell walls from portions of the egg apparatus occurs after pollination. This loss occurs between degenerate synergid and central cell and between central cell and egg. An intrusion of central cell cytoplasm into the degenerate synergid takes place (Figs 3, 4, 5). The cytoplasms of the 2 cells are separated only by membranes but their ultrastructure is vastly different. Partial loss of wall between egg and central cell also occurs (Fig. 5). The embryo sac of barley is now capable of facilitating pollen tube penetration and transmission of male gametes.

The pollen tube enters the barley embryo sac through the filiform apparatus of the degenerate synergid (Fig. 6); the pollen tube grows into the degenerate synergid and discharges its contents, including 2 sperm cells, a vegetative nucleus, and many pollen tube vesicles (Cass, Peteya, 1979). One sperm nucleus fuses with the polar nucleus prior to the fusion of the second sperm nucleus with the egg nucleus. The sperm nucleus identified in Fig. 7 will fuse with the egg nucleus as the other fusion had already occurred in this embryo sac. The male gametes of barley are cellular (Cass, 1973; Cass, Karas, 1975) and

PLATE I



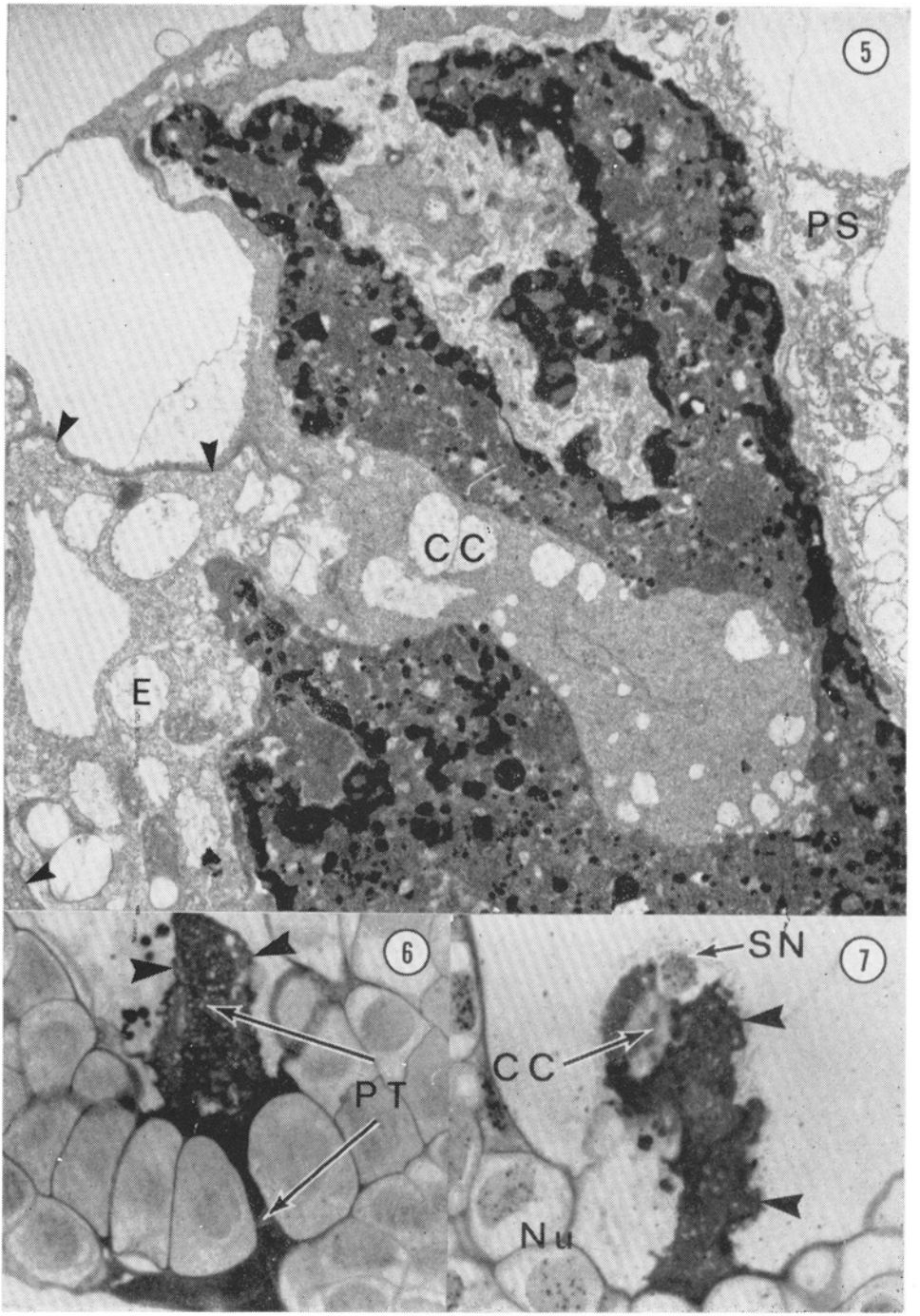

5
PS
CC
E
6
PT
7
SN
CC
Nu

Fig. 5. Electron micrographs of egg apparatus cells after pollination — part of central cell embayment (CC) and its relation to profile of egg cytoplasm (E) separated from it by membrane (arrowheads). Persistent synergid appears to right. 10.300 ×

Figs 6, 7. Brightfield micrographs taken at time of fertilization. Fig. 6. shows pollen tube (PT) in micropyle and degenerate synergid (unlabeled arrows). Profile of degenerate synergid shown by arrowheads. 1150 ×. Fig. 7 is autoradiograph of sperm nucleus (SN) in degenerate synergid (arrowheads) apparently approaching central cell embayment (CC). Nucellar cells (Nu) also labeled with H^3-actinomycin D shown to left. 1150 ×

are deposited in the embryo sac as cells (Cass, Jensen, 1970). Male cytoplasm enters the egg as indicated by transmission of cytoplasmic virions by barley sperm cells (Carroll, Mayhew, 1976).

DISCUSSION

This study provides a possible basis for understanding the process of male gamete transmission during fertilization in flowering plants. Degeneration of one synergid and modification of the egg/central cell boundary may facilitate sperm transfer among egg apparatus cells through selective wall removal. Passage of the naked sperm cells from cell to cell as a result of this wall removal involves passage through membranes. Specific membrane contact between sperm cell and the embryo sac cell it is destined to enter might permit or preclude passage.

Acknowledgments

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