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# Cytoskeletal and Nuclear Behavior During Female Gametophyte Development and Fertilization in Angiosperms

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## INTRODUCTION

The basic events of embryo sac development and fertilization have been extensively studied using classical techniques in light microscopy and electron microscopy. The megaspore mother cell produces four megaspores after meiosis. Only one chalazal megaspore survives and then goes through three mitotic divisions giving rise to a 7-celled embryo sac. However, the information concerning nuclear positioning and anchoring, nuclear division, cell wall and filiform apparatus formation and its correlation to the changes of mcirotubular cytoskeleton is still scarce due to the inaccessibility of megasporocyes and megagametophytes.

In most of the species studied, the prominent features during fertilization include the degeneration of one of the synergids, its subsequent penetration by the pollen tube, and release of the male gametes which subsequently migrate and fuse with the female target cells. The elements of this fertilization system are well described, but the organization and possible involement of the cytoskeleton of the embryo sac have only recently been examined. The exclusion of the sperm cytoplasm before fusion is not yet completely understood.

To obtain a better understanding of the microtubular and nuclear behavior during the embryo sac development and the involvement of cytoskeleton during fertilization, we have characterized the changes of oganellar DNA and the role of the microtubular cytoskeleton during megasporogenesis and megagametogenesis in maize and structural and cytoskeletal changes during fertilization in *Nicotiana tabacum*.

## RESULTS AND DISCUSSION

Embryo sac development is initiated by the meiotic divisions of the megaspore mother cell. The cytoplasmic organelles are predominantly distributed in the chalazal region of the megasporocyte reflecting the early polarity of the cell which confirm the previous reported premeiotic establishment of megas-porocyte polarity. After meiosis I the two nuclei of the megasporocyte migrate to the opposite poles of the cell which elongates to a large extent. By the completion of meiosis, the megasporocyte has produced four megaspores, among which only the chalazal-most megaspore survives while the three other megaspores degenerate. The organellar DNA of the megaspore is mainly perinuclear. The first mitosis of the surviving megaspore gives rise to the two-nucleate embryo sac. The polarization of he microtubules and organellar DNA becomes conspicuous. Dense microtubule bundles in the perinuclear region of the mcropylar end of the embryo sac form an anastomosing network extending from the nucleus to the cell cortex. The micropylar nucleus is surrounded by a dense accumulation of organellar DNA and perinuclear microtubules, while those in the chalazal end are present to a lesser extent. Although the precise function of the these perinuclear microtubules is unclear, it is likely that each pole of microtubules claims a region of cytoplasm in which the microtubules extend and maintain one pattern of polarity. The polarity of DNA-containing organelles is apparently established in the late two-nucleate embryo sac with the DNA-containing organelles predominantly surrounding the micropylar nucleus. Because the young egg cell con-

tains a significant number of the DNA-containing organelles after cellularization of the embryo sac, it appears that this early event in the determination of the reproductive cells occurs in the two-nucleate stage, although cellular differentiation is delayed until cytokinesis at the eight-nucleate stage.

The initiation of the second mitosis is followed by simultaneous nuclear divisions at both the micropylar and chalazal poles. At telophase of the second mitosis the phragmoplast at the micropylar end is oriented parallel to te long axis of the embryo sac and is nearly perpendicular to the transverse orientation of the chalazal phragmoplast. Consequently, the plane of alignment of the pair of sister nuclei at the micropylar pole is nearly a right angle to that of sister nuclei at the chalazal pole. Simultaneous nuclear division also occurs in the four nuclei at the third mitosis. At cytokinesis of the third mitosis phragmoplasts ar simultaneously formed between the eight nuclei at the micropylar and the chalazal poles, indicating the location of future cell walls . The disturbance of the positioning of the female gametophyte nuclei and their spatial relationship during cell formation and differentiation embryo sacs carrying the maize indeterminate gametophyte (ig) mutation results in the formation of unanticipated cell types. This suggests that the positioning of the nuclei by control of mitotic spindle orientation and by control of unclear migration is fundamentally related to the determination of cell fate and cell differentiation in the developing embryo sac. After cellularization, two synergids and one egg cell form at the micropylar pole, three antipodal cells form at the chalazal pole, and the central cell occupies the center of the embryo sac. Numerous longitudinally aligned microtubule bundles are localized at the synergids and the antipodal cells, while sparse transverse microtubule arrays are seen in the central cell, mainly at the cortical region. The antipodal cells continue to divide and produce variable numbers of cells at the chalazal pole until embryo sac maturity.

Before fertilization, the synergid is a highly polarized cell with dense longitudinally aligned arrays of microtubules adjacent to the filiform apparatus at the micropylar end of the cell associated with major organelles. However, it is evident upon examination of cryofixed material that one of the synergids displays a dramatic morphological change after pollination, which begins to degenerate before pollen-tube arrival with breakdown of the plasma membrane and the larger chalazal vacuole delayed until the penetration of the pollen-tube. The electron density of cytoplasmic organelles increases and the nucleus becomes distorted. Abundant electrondense material extends from the degenerated synergid into intercellular spaces at the chalazal end of the synergid and between the synergids, egg and central cell. Rhodamine-phalloidin and immunogold labeling revealed that this electron-dense material contains abundant actin. Actin in the synergid becomes locally intense and particularly conspicuous at the chalazal end of the dengerated synergid, forming two "corona"-like bands at the inter-faces between the egg, central cell and synergid. The location of myosin-like proteins in pollen tube and on the surfaces of generative cells and vegetative nuclei presents the possibility that actomyosin interactions act as a controlling factor in the deposition, positioning and fusion of male gametes during fertilization. The sperm cell, which are believed to lack actin, may therefore be passively conveyed by means of the superficial myosin of the sperm cells and the actin coronas of the embryo sac. This actin is part of a mechanism in the egg apparatus which apppears to precisely position and facilitate the access of male gametes to the egg and central cell for fusion.

Upon pollen-tube arrival, the male gametes are released through a terminal aperture into the degenerate synergid. Cellular organelles remain largely intact after polen tube discharge. This suggests that the onset of synergid degeneration occurs before the arrival of the polen tube, but the breakdown of the cell occurs only after pollen tube penetration. Unfused sperm cells within the degenerate synergid appear to be strongly modified by their passage in and discharge from the pollen tube. The exterior of the sperm cell is delimited by a single membrane. Apparently once pollen tube discharge occurs, the inner pollen tube plasma membrane is stripped off and releases the sperm and vegetative nucleus triggering the disassociation of the male germ unit and allowing the gametes to separate and follow their respective fates. Before the gametic fusion, a few heritable organelles, apparently mitochondrial, are evident in the cytoplasm of the sperm, but these appear to be enclosed within ER inclusion. Dictyosomes, ER, and a rounded nucleus are

also prominent features prior to gametic fusion. Vacuolization and the "rounding" of sperm cells occur only after the sperm cells are deposited in the degenerate synergid, but not during normal growth within the pollen tube. The exclusion of sperm cyto-plasm and explosion of its single membrane may represent a crucial phase in the preparation of the sperm for fusion.