Embryo sac development in *Arabidopsis thaliana*

II. The cytoskeleton during megagametogenesis

**Abstract** The microtubular and actin cytoskeletons have been investigated during megagametogenesis in *Arabidopsis thaliana* using immunofluorescence labelling of isolated coenocytic and mature embryo sacs. We found both actin and microtubules (MTs) to occur in abundance throughout megagametogenesis and in all constituent cells of the mature embryo sac. During many stages, the patterns of distribution of these cytoskeletal elements are congruent and may prove to be co-aligned. Many changes in the arrays of MTs and microfilaments take place and indicate varying roles of the cytoskeleton in the different stages and cell types of megagametogenesis. Two major populations of MTs recur throughout embryo sac formation: (1) Elaborate nuclear-based networks are found during the two-nucleate and four-nucleate developmental stages as well as in the egg cell. These arrays may function in positioning the nuclei. (2) Cytoplasmic MTs in longitudinal orientation in the two-nucleate embryo sac, synergids and part of the egg cell, or in a reticulate pattern in the four-nucleate embryo sac, egg and central cell probably participate in organization of the cytoplasm. Synergid MTs converge at the filiform apparatus. Preprophase bands of MTs are absent throughout megagametogenesis but phragmoplast arrays occur during cellularization of the embryo sac. Well developed arrays of cortical MTs are restricted to the antipodal cells. A large concentration of MTs in the part of the egg cell adjacent to the synergids is well placed for being involved with sperm cell movement within the degenerative synergid. On the basis of the morphology of the cytoskeleton, we concur with views that the shape of megagametophyte is largely determined by the surrounding tissues, including the integumentary tapetum.

**Key words** *Arabidopsis thaliana* · Megagametogenesis Cytoskeleton · Microtubules · Actin

**Introduction**

Embryo sac formation is crucial to the reproductive success of flowering plants, nevertheless our knowledge of its mechanism is quite incomplete. The cytoskeleton is one aspect of embryo sac development which has been underexplored (Webb and Gunning 1993), although its contribution to essential cellular processes in other cell types is well known. *Arabidopsis thaliana* is a well defined model system offering great potential for expanding our studies of normal development to investigating the cytoskeleton in reproductive developmental mutants which have been isolated (e.g., floral developmental mutants, Bowman et al. 1989; embryo-associated mutants, Meinke 1985, 1991, Mayer et al. 1991; female sterile mutants, Robinson-Beers et al. 1992). Megagametogenesis in *A. thaliana* follows the monosporic Polygonum type (Misra 1962; classification system of Maheshwari 1950). Details of the cytology are well established (e.g., Misra 1962; Poliakova 1964; Mansfield et al. 1991; Bowman 1993; Webb and Gunning 1993). Briefly, the functional megaspore divides mitotically to form the two-nucleate, four-nucleate, and, subsequently the eight-nucleate embryo sac. Dominant, centrally-located vacuoles are present by the end of each of these stages of development. Nuclei then migrate to specific positions at the poles of the coenocytic cell before cellularization occurs to give a seven-cell megagametophyte. The micropylar part of the embryo sac houses two synergids and the egg cell. Three antipodal cells are found at the chalazal end. A large central cell occupies most of the volume of the mature embryo sac and is binucleate, one nucleus having come from each end of the coenocyte. In *A. thaliana* the polar nuclei

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