Acta Bot. Neerl. 37(3) September 1988, p. 387–394

The characteristics and fate of the soybean inner nucellus

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SUMMARY

Development of the soybean inner nucellus is described. The inner nucellus is a distinct region composed of cells that stain darkly with toluidine blue oxide and have thickened walls when compared with the remaining nucellus. This tissue region becomes apparent during early megasporogenesis and completely surrounds the expanded megasporocyte, megaspores and 2- and 4-nucleate megagametophytes. By the time the megagametophyte is cellular the inner nucellus is restricted to the chalazal end of the megagametophyte. The cells of the inner nucellus of soybean exhibit a normal ultrastructural appearance with an elevated concentration of ribosomes and numerous plasmodesmatal connections among themselves, to cells of the outer nucellus and to the developing and cellular megagametophyte. Our observations suggest that the inner nucellus of soybean may provide metabolites for the developing megagametophyte.

Key-words: megagametogenesis, nucellus, nutrition, ovule, soybean.

INTRODUCTION

During certain developmental stages the soybean nucellus contains a distinct cellular region as yet unreported (Pamplin 1963; Prakash & Chan 1976; George *et al.* 1979; Folsom & Peterson 1984; Kennell & Horner 1985; Folsom & Cass 1986). As this extensive region of cells cannot be equated with crushed nucellar cells, which are often seen immediately adjacent to the mature embryo sac wall, and does not correspond to any nucellar modifications discussed by Maheshwari (1950), we refer to it as the 'inner nucellus'.

It has been demonstrated that the choice of fixative influences which cellular components are preserved (O'Brien *et al.* 1973). A review of papers published on soybean megasporogenesis and megagametogenesis shows that only one study (Kennell & Horner 1985) utilized the non-coagulative fixative, gluteraldehyde. All the others (Pamplin 1963; Prakash & Chan 1976; George *et al.* 1979) used a variety of fixatives which are coagulative in nature. Coagulative fixatives have been shown to destroy many cellular components during fixation (O'Brien *et al.* 1973).

MATERIAL AND METHODS

Plants of soybean, *Glycine max* (L). Merr. 'Gnome', were grown in the University of Alberta Phytotron. Preanthesis flowers of various developmental stages were dissected,

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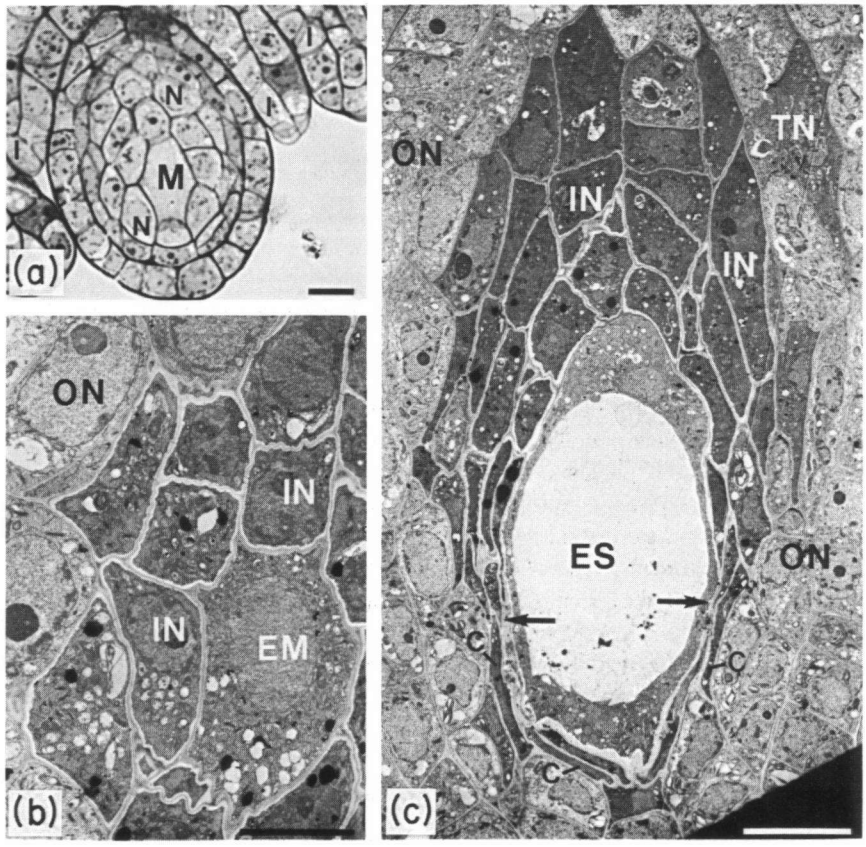

Fig. 1. Soybean nucellar tissue at different development stages. (a) Megaspore mother cell of soybean before development of the inner nucellus stained with TBO. Bar indicates 10 μm. (b) Expanded soybean megaspore mother cell and inner nucellar cells. TEM. Bar indicates 5·0 μm. (c) Destruction of the inner nucellus around the micropylar base of a 4-nucleate megagametophyte (arrows). TEM. Bar indicates 10 μm. (A) antipodal cell; (C) crushed inner nucellar cell; (CC) central cell; (EM) expanded megaspore mother cell; (ES) embryo sac; (I) integument; (IN) inner nucellar cell; (M) megaspore mother cell; (N) nucellus; (ON) outer nucellar cell; (P) plasmodesmata; (TN) transitional nucellar cell.

ovules fixed, embedded, sectioned and observed according to the protocol of Folsom & Cass (1986). Material for light microscopy came from that fixed and embedded for electron microscopy. Sections 1–2 μm were cut with glass knives, fixed to slides at 80°C and stained with toluidine blue oxide (TBO) (Yeung 1984).

RESULTS

During the later stages of megaspore mother cell expansion, cellular changes result in a population of nucellar cells very different from those seen in the rest of the soybean nucellus. Based on their position, these cells are collectively referred to as the inner nucleus (compare Fig. 1a and b). Once formed, inner nucellar cells remain intact until they are destroyed by megagametophyte expansion which begins around the micropylar base of the embryo sac and proceeds chalazally (Fig. 1c). The destruction of the inner nucellus appears to begin with development of the 4-nucleate embryo sac. Cells of the inner

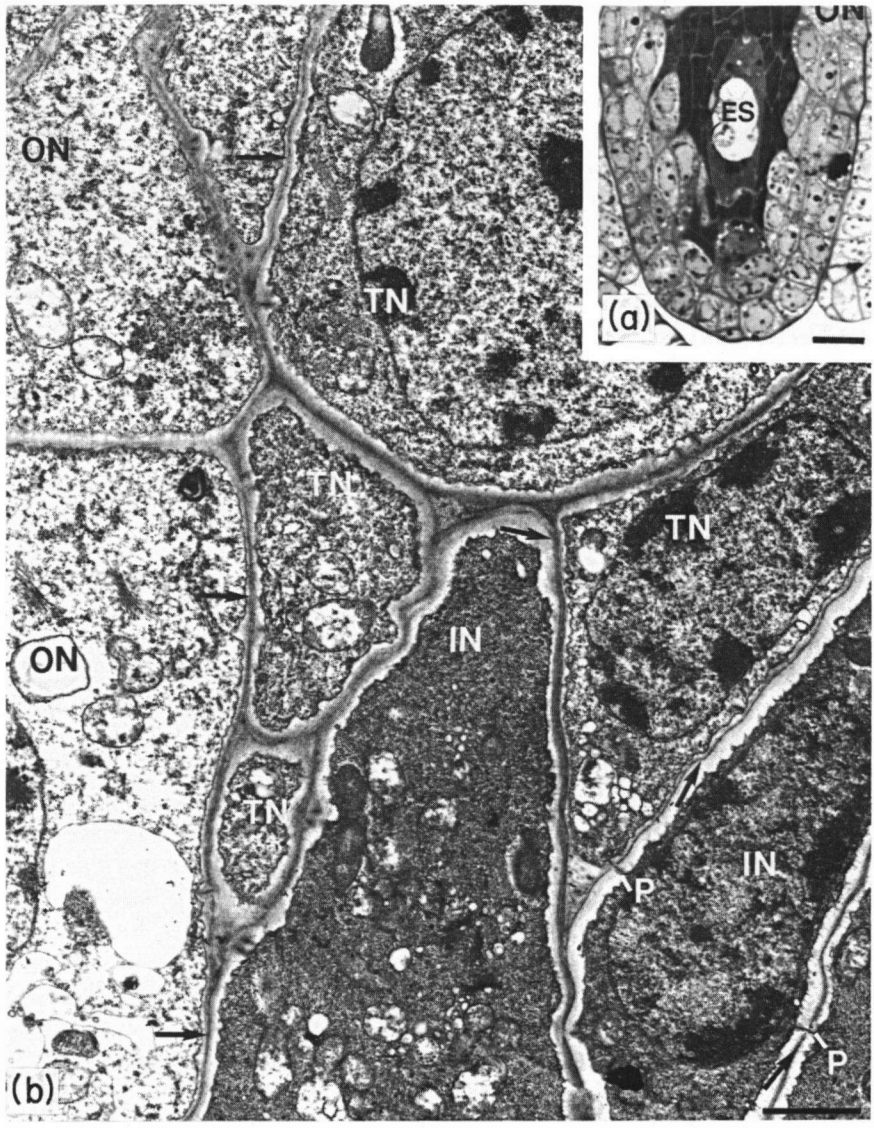

Fig. 2. Characteristics of soybean inner nucellar cells. (a) 2-nucleate embryo sac stained with TBO showing the inner and outer nucellar cells. Bar indicates 10 μm. (b) Inner, outer and transitional nucellar cells. Arrows point to middle lamella. TEM. Bar indicates 1·0 μm. For abbreviations see legend to Fig. 1.

nucellus stain intensely with toluidine blue oxide (TBO) (Fig. 2a) and ultrastructural observations show that these cells exhibit a dense ribosomal granularity when compared to other cells of the nucellus (Fig. 2b).

Increased wall thickness is also shown by inner nucellar cells. When ultrastructural comparisons are made between inner nucellar cell walls and those of other nucellar cells, the uniform increased thickness of inner nucellar walls is evident (Fig. 2b). This is a characteristic of all inner nucellar cells; however, there are cells in the region where the inner nucellus is contiguous with the rest of the nucellus, which appear to be transitional in

nature, showing walls thickened only in certain regions (Fig. 2b). These transitional cells often possess cytoplasm with an elevated ribosomal concentration when compared to outer nucellar cells. However, the density of these transitional cells is still less than that seen in inner nucellar cells (Fig. 2b). After the inner nucellus is formed transitional cells only appear to occur at the chalazal end of the nucellus (Fig. 1c). Walls of inner nucellar cells contain numerous plasmodesmata (Fig. 2b). Plasmodesmata are also seen in the expanded megaspore mother cell (Fig. 3a) and coenocytic megagametophyte wall (Fig. 3b). In later stages of embryo sac development, plasmodesmata only occur in the megagametophyte wall connecting antipodals to inner nucellar cells (Fig. 3c).

DISCUSSION

Many specialized cellular regions have been described in the nucellus of various plants (e.g. the epistase, hypostase and archesporium). Maheshwari (1950) states that the archesporium is a group of large dense cells from which the megaspore mother cell develops. He defines the epistase as a tissue found in the micropylar portion of the nucellus and the hypostase as a tissue which develops between the megagametophyte and chalazal vascular trace of the ovule. As the inner nucellus does not form until after the megaspore mother cell develops and expands, it appears unlikely to be part of the archesporium. Neither a hypostase nor epistase has been reported to occur in any of the Fabaceae before fertilization. The inner nucellus which develops around the megaspore mother cell is not limited to either the chalazal or micropylar portion of the nucellus and does not occur near the chalazal vascular trace. Therefore, the inner nucellus cannot be either an epistase or hypostase.

The metachromatic stain toluidine blue oxide (TBO) can be used as a reliable indicator for the presence of nucleic acids (Feder & Wolf 1965). When TBO staining of the inner nucellus (Fig. 2a) is compared with ultrastructural observations (Fig. 2b), it is evident that the elevated density of the inner nucellus is mainly due to the presence of a high ribosomal concentration. Whether the inner nucellus occurs only in soybeans or also in other members of the bean family and what is (are) the factor(s) responsible for inner nucellar differentiation remains an open question. The observation that the inner nucellar cells also occur in cellular layers far removed from the embryo sac wall suggests that these cells are simply not degenerate nucellar cells but rather typical outer nucellar cells which are stimulated to undergo differentiation well in advance of the expanding embryo sac. Further support for the concept that inner nucellar cells are products of cellular differentiation comes from observations that certain cells at the boundary between the inner nucellus and the remainder of the nucellus are transitional, intermediate in cytoplasmic density and often show areas of thickened walls (Fig. 2b).

No nucellar modifications of this type have been reported to occur before fertilization in soybean (Pamplin 1963; Prakash & Chan 1976; George *et al.* 1979; Folsom & Peterson 1984; Kennell & Horner 1985). Densely staining crushed nucellar cells, in the cell layer immediately adjacent to the mature soybean embryo sac wall, have been reported before (Folsom & Peterson 1984). These cells contain an amorphous osmiophilic material similar in density to that found in the degenerate synergids of cotton (Jensen & Fisher 1968) which is not similar to that found in inner nucellar cells. In the nucellus of *Oenothera* it has been reported that the process of cellular degeneration and crushing involves cytoplasmic changes including dilatations between nuclear membranes and an infolding of smooth endoplasmic reticulum to form digestive vacuoles (Noher de Halac 1980). The

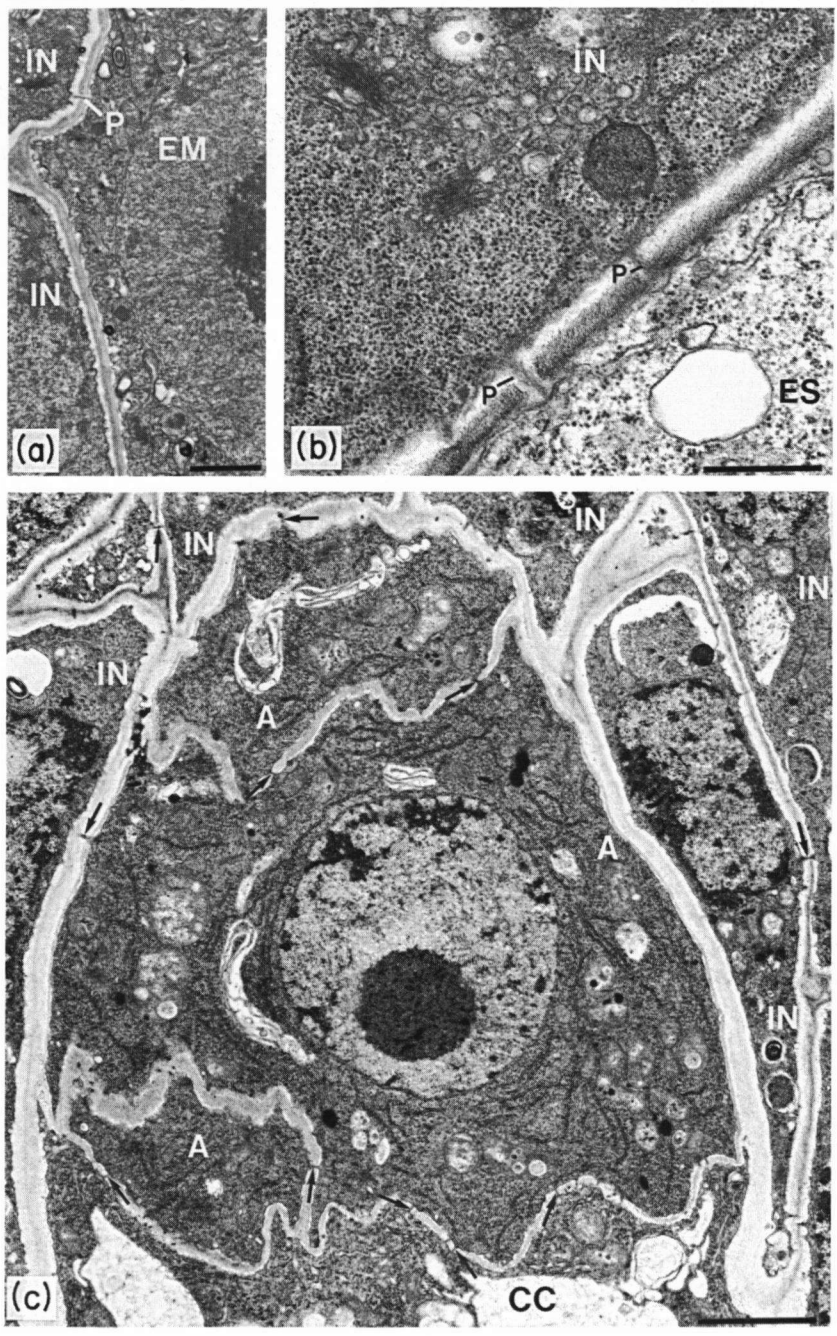

Fig. 3. Symplastic pathways associated with the developing soybean megaspore mother cell and embryo sac. (a) Plasmodesmata between inner nucellar cell and expanded megaspore mother cell. TEM. Bar indicates 1·0 μm. (b) Plasmodesmata between inner nucellar cell and 4-nucleate embryo sac. TEM. Bar indicates 0·5 μm. (c) Plasmodesmata (arrows) between inner nucellar cells and antipodals of a cellular megagametophyte. Plasmodesmata (arrows) are also seen in walls between antipodal cells and in walls common with the central cell. TEM. Bar indicates 2·0 μm. For abbreviations see legend to Fig. 1.

ultrastructure of soybean inner nucellar cells is not similar to that reported by Noher de Halac (1980) in degenerate *Oenothera* nucellar cells. Furthermore, soybean inner nucellar cells do not resemble degenerate nucellar cells seen during early embryo sac development in *Capsella* (Schulz & Jensen 1986).

As the expanded megaspore mother cell, megaspores, persistent megaspore, 2- and 4-nucleate megagametophytes and chalazal region of the cellular megagametophyte are all enclosed by and symplastically connected to the inner nucellus with plasmodesmata, it seems likely that these cells have a nutritional function. This idea is supported by observations that show that the only plasmodesmata in the expanded megaspore mother cell or megagametophyte wall occur in regions common with inner nucellar cells. Support for this view of inner nucellar function also comes from the fact that ultimately the only symplastic route for nutrients to enter developing embryo sac cells occurs *via* the inner nucellus. As cell walls may serve as a transport pathway (Lüttge & Higinbotham 1979), the increased thickness of inner nucellar cell walls could facilitate transport to the developing megaspore mother cell, megaspores and megagametophyte. It is not until the antipodals senesce and the megagametophyte expands chalazally that the last cells of the inner nucellus are destroyed. When this occurs the remaining plasmodesmata between the embryo sac and inner nucellar cells are destroyed and the megagametophyte becomes symplastically isolated from all nucellar cells.

We can only speculate why the inner nucellus has not been reported on before. As most of the studies on soybean embryo sac development were done with coagulative fixatives (Pamplin 1963; Prakash & Chan 1976; George *et al.* 1979), which have been shown to destroy cell cytoplasm (Baker 1958; O'Brien *et al.* 1973; Mersey & McCully 1978), the increased ribosomal concentration of inner nucellar cells would be difficult to detect. Therefore, it is not surprising that the existence of an inner nucellus has not been reported before.

ACKNOWLEDGEMENTS

We would like to thank Dr G. W. Scott, Director of the Surgical-Medical Research Institute at the University of Alberta, Mrs E. Schwalt and Mr G. Higgs for their help with the TEM. We also wish to thank Drs R. J. Martin and R. L. Cooper, both of The Ohio Agricultural Research and Development Center, for their gift of the Gnome soybean seeds. Support for this research was provided by grants to D.D. Cass from NSERC (A-6103) and the University of Alberta (CRF 755-48686).

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