Ultrastructure of Antipodal Cells of Rice (*Oryza sativa*) before Anthesis with Special Reference to Concentric Configuration of Endoplasmic Reticula

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Abstract : Before anthesis, rice antipodal cells are located between nucellar cells and a central cell, and have a large-sized lobed nucleus and many cell-wall ingrowths abutting the nucellar cells. An outstanding feature in the cytoplasm is the appearance of a multilayered, concentric configuration in endoplasmic reticula. They are constituted of several rings which enclose a component of cytoplasm or a plastid in the center. Two or three rings are connected with each other through a small joint, and as a result, a continuous lumen is ensured. These complicated figures of endoplasmic reticula probably come apart just after anthesis and rearrange into a great number of singular cisternae, of which features have been illustrated. From these circumstances, it is concluded that the configuration of endoplasmic reticula in antipodal cells strictly depends upon the developmental stages of the embryo sac. Furthermore, the dilation of lumens is observed on the singular cisternae located near the cell wall separating the central cell. The deposition and dilation of the singular segments are meaningful in relation to the nutrient transfer from the antipodal cells into the central cell. Finally, the higher activity of rice antipodal cells is indicated from the ultrastructural viewpoint. Additionally, the configurational change of endoplasmic reticula is discussed in the context of metabolic activities in the antipodal cells of rice and other plants.

Key words : Antipodal cell, Concentric configuration, Endoplasmic reticulum, Lobed nucleus, *Oryza sativa*, Ultrastructure.

開花直前のイネ反足細胞の微細構造とくに同心円状の小胞体に注目して : 前田英三・三宅 博* (東京農業大学生物産業学部・*名古屋大学農学部)

要 旨 : イネの反足細胞は開花前に、裂片状の部分をもつ大きな核と多くの細胞壁突起をもっている。同心円環状の小胞体の存在が、その細胞質の特徴である。同心円状小胞体の中心には、細胞質の一部やプラスチドが見られる。また環状構造の内腔は、ときに小さな接合部で融合している。開花後に小胞体が多く的小片となることから、開花すなわち受精の前後で小胞体の構造が顕著に変化すると考えられる。胚嚢内中心細胞に近い反足細胞の細胞質内、およびその細胞壁に接して内腔の肥大した小胞体切片の見られることから、反足細胞から中心細胞への物質転流機構に対する小胞体の関与が推測された。

キーワード : イネ, 小胞体, 同心円状構造, 反足細胞, 微細構造, 裂片状細胞核。

Development of embryo sacs and embryos in rice plants has attracted our attention and led us to examine early important events in the course of seed formation and also to scrutinize the reality of somatic embryogenesis in rice callus cultures¹⁵⁾. We published the first study along these lines in 1990, when the ultrastructure of an egg apparatus was examined⁷⁾. A recently published paper⁸⁾ concerned the ultrastructure of rice antipodal cells after anthesis, that is, at the fertilization period, and demonstrated the transfer routes of nutritives into and within embryo sacs. It has been indicated that the antipodal cells take up the nutritives from nucellus cells exclusively through cell-wall ingrowth and make them move into a central cell via an apoplastic route.

The present study aims to clarify the ultrastructure of rice antipodal cells before anthesis and to compare it with cellular morphology of the antipodals immediately after anthesis. Morphological evaluation was made by means of transmission electron microscopy. The antipodal apparatus before anthesis comprises large-sized adjacent cells, in which a wheel-like morphology of lobe-shaped nuclei and concentric configuration of multilayered endoplasmic reticula are characteristically discerned.

Materials and Methods

Rice (*Oryza sativa* L. cv. Hatsuhoshi) ovaries, which will come into flower within the next 24 h, were removed from the panicles of the plants maintained in culture pots, fixed according to an aldehyde-osmium schedule

and embedded in epoxy resin. Detailed procedures employed in this study were the same as those described previously^{7,8,12}. Ultrathin sections were viewed at 100 kV on an Hitachi H 600 transmission electron microscope.

Results

Before anthesis, rice antipodals proliferate into a mass of enormously large cells and are parietally-positioned along the nucellus. The giant antipodal cells have various organelles in ribosome-rich cytoplasm with weak vacuolation and prominent ingrowth of cell wall along the nucellar cells. There are large nuclei irregularly shaped, strongly developed endoplasmic reticula, numerous mitochondria and a small number of plastids. Singular cisternae of endoplasmic reticula are frequently located near the cell wall separating the antipodal cells from the central cell. A striking feature is the frequent appearance of endoplasmic reticula showing a concentric type. Occasionally a convoluted type of endoplasmic reticula is seen.

Fig. 1 shows an antipodal cell situated between the central cell and nucellar cells. The antipodal cells display a uniform distribution of various organelles, that is ribosomes, rough endoplasmic reticula, mitochondria and plastids. There is a large-sized nucleus, which has a nucleolus with nucleolar vacuoles but contains a very small amount of heterochromatin. The nuclear envelope is slightly invaginated and gives rise to small extrusions which surround a small component of cytoplasm containing the short and singular cisternae of endoplasmic reticula. At right of Fig. 1, the presence and appearance of a concentric shape in rough endoplasmic reticula are definitely seen in the cytoplasm, including many of mitochondria with fairly-developed cristae and some plastids with a few lamellae. Dictyosomes present in the vicinity of the central cell and sometimes singular cisternae are in contact with the cell wall abutting the central cell (as shown with arrows).

Fig. 2 indicates the component of cytoplasm surrounded by the extrusions, which is produced by vigorous invagination of a lobed nucleus. Each lobed part of a nucleus has a nucleolus with several vacuoles and is connected by small bridges of nucleoplasm. Owing to the invagination and the extrusion,

the envelope of lobed nucleus is extraordinarily sinuous at the side facing the cytoplasm component. Thus, a wheel-like morphology of nucleus is displayed. This structure is almost similar to that illustrated in the previous paper⁸) and also suggests an intensive activity allowing the materials in the lobed nucleus to transfer into the cytoplasm component surrounded by the nucleus. Nuclear pores are numerous on the nuclear envelope located at the side facing the cytoplasm component. Osmiophilic globules are common in the vicinity of the nuclear envelope.

The distal ends of the nuclear extrusions are elongated and make small oblong parts, of which figures appear to be pinched off from the lobed nucleus and finally to be released as separate pieces into the cytoplasm. The nucleoplasm in the distal ends is denser than other parts (arrows). The fate of the distal ends is interesting from the viewpoint of the occurrence of endoplasmic reticula. In the cytoplasm component surrounded by nuclear extrusions, mitochondria with well-developed cristae, short segments of dilated endoplasmic reticula and a high density of free ribosomes are present.

The behavior of endoplasmic reticula with attached ribosomes is very fascinating. Frequently they have extensively developed cisternae showing parallel and concentric configuration. In the center of the concentric type, there is a component of cytoplasm and plastids with or without starch grains. Sometimes, a small bridge makes up a fusion of the lumens, which are inner compartments of two rings forming a part of the concentric configuration.

Fig. 3 shows two figures of concentric cisternae with attached ribosomes. They are separated from each other, one encloses an elongated plastid of an ill-developed state in the center and the other encloses small-sized components of cytoplasm. They are connected by the presence of an elongated singular cisterna of endoplasmic reticulum (an arrow). An asterisk shows a fusion of lumens in three rings forming a concentric feature, by which the inner compartments are united into a continuous body. The lumens have electron transparent contents. In the vicinity of these figures, there are dictyosomes with developed vesicles and a large number of well-developed mitochondria. Osmiophilic globules are scattered

in the cytoplasm and often appear in the lumens of endoplasmic reticula.

Disposition of singular cisternae commonly occurs in the antipodal cells but are more readily noticed in the cytoplasm adjacent to the central cell. They are rarely grouped. In Fig. 4, which shows antipodal cytoplasm near the central cell, endoplasmic reticulum segments are seen to be rather short. Frequently the profiles of the segments are closely parallel with the plasma membrane of the antipodal cell and the lumens are dilated showing the contents of a thin, fibrous aspect.

More clearly, this situation can be seen at a high magnification in Fig. 5. There are some single profiles of endoplasmic reticula, the lumens of which are dilated and contain some materials in flocculent appearance. A microbody enveloped with a single membrane is present showing a round inclusion in relatively dense matrix (asterisk).

Discussion

Present study makes an ultrastructural description of rice antipodal cells before anthesis. Cells of giant size compose the antipodal mass. The cell has an elaborate structure, including a prominent nucleus. Sometimes the lobed nucleus shows a wheel-like shape in profile. The wheel-like morphology of nucleus remains in mysterious nature, but the complicated figures are implying the activity, by which the amount of endoplasmic reticula is increased. The antipodal cell has a dense cytoplasm enriched with various organelles. The ground cytoplasm is filled with ribosomes, endoplasmic reticula and mitochondria. The presence of these organelles is an indicator of vigorous cell activity at the point of protein synthesis and energy supply.

It has been mentioned that the rice antipodal cells hold their divisions before and even after fertilization, and result in a multicellular mass. Further, the presence of concentric endoplasmic reticula has been indicated in the cell³⁾. Barley antipodal cells, together with nuclei and nucleoli, increase several times in size with the advance of time, before and after fertilization, and small intrusions of the nuclear membrane are formed in them⁴⁾.

Large concentric aggregates of the rough endoplasmic reticula have been described in several reports. They appear in the ovaries of

Aizoaceae species⁶⁾, the male-sterile anther cells of *Beta vulgaris*¹¹⁾, the vascular bundle cells of ungerminated rice coleoptiles¹³⁾, the parenchymatous cells of rice scutella cultured in vitro^{9,10,14)}, the root cells of *Zea mays* under the drought and cold stresses²⁾, the radicle cells of mustard seeds¹⁾, the root cells of the mustard seedlings under osmotic stress¹⁾ and the root-cap cells in *Zea mays* treated by chlorsulfuron as a herbicide⁵⁾. In barley antipodal cells, there are some cisternae annularly arranged. In addition, the concentric aggregates enclose cytoplasmic components¹¹⁾ and various organelles in the center, that is, plastids¹⁾, lipid bodies^{4,9,10,14)}, oleosomes¹⁾, derivatives of the Golgi apparatus or vacuoles⁶⁾, protein bodies¹⁾ and electron dense deposits⁹⁾.

It is confirmed in our examination that the endoplasmic reticula in rice antipodal cells before anthesis are largely of the rough type, often with stacked cisternae, and sometimes construct a concentric morphology. Several rings included in concentric configuration of endoplasmic reticulum are linked to each other by a small bridge. It is unclear whether these complexes of endoplasmic reticula reflect the enhanced activity or the inactive state of protein synthesis, since they have been discerned in both very active cells and resting cells, as shown in the above mentioned publications.

As was pointed out in an earlier paper⁸⁾, short or long linear profiles of singular cisternae, sometimes showing a circular figure, are present in rice antipodal cells after anthesis. Before anthesis, however, endoplasmic reticula in the cytoplasm of rice antipodal cells often appear in a multilayered, concentric configuration. Consequently, the morphological change of endoplasmic reticula is discernible at the different stages of rice antipodal cells. After anthesis, the concentric configuration is probably transformed into a great number of single-stranded cisternae. It may be pointed out that an important change in metabolic events occurs in antipodal cells during the development of the embryo sac. Unfortunately, not much information is available regarding the conformational change of endoplasmic reticulum.

Metabolic information underlying these rearrangements is still lacking. Biochemical

Abbreviations

CC : central cell, Ci : cisterna, CR : concentric endoplasmic reticulum, CW : cell wall, Cy : cytoplasm, D : dictyosome, IW : ingrowth of cell wall, M : mitochondrion, N : nucleus, Nc : nucellus, NEv : nuclear envelope, No : nucleolus, NP : nuclear pore, NV : nucleolar vacuole, P : plastid, R : rough endoplasmic reticulum.

Explanation of Figures

- Fig. 1. Antipodal cell abutting a central cell in one side and nucellar cells in the other side. Arrows show singular cisternae contacted to the cell wall separating a central cell.
- Fig. 2. Various figures of inclusions in the cytoplasm component surrounded by lobed parts of a nucleus. Arrows show distal ends of the nuclear extrusions.
- Fig. 3. Two figures of concentric endoplasmic reticula enclosing an elongated plastid or a component of cytoplasm. Concentric figures are connected by a long, singular cisterna shown by an arrow. An asterisk shows a fusion of lumens.
- Fig. 4. Disposition of cisternae in the cytoplasm adjoining the central cell.
- Fig. 5. Higher magnification view of cisternae in the vicinity of the cell wall adjoining the central cell. Arrows show the dilated cisternae. An asterisk shows the morphology of a microbody.

events raised by configuration of endoplasmic reticula are not possible to be analyzed by the fraction of microsomes ordinarily prepared from rough endoplasmic reticula. If the different fractions of microsomes are separately acquired through any relevant method from each of concentric figures and singular segments, we would be able to examine and realize the mechanism involved in the configurational change of endoplasmic reticula during the development of rice antipodal cells.

On the other hand, dilated singular cisternae are preferentially distributed along the cell wall abutting a central cell. This is suggestive of a transfer facility of the nutritives. Additionally, it has been indicated that in the central cell of rice embryo sac, small segments of singular cisternae are in close contact with the plasma membrane, which is running in parallel with the plasma membranes of an egg cell and synergids⁷⁾. This is probably to visualize transfer activity of singular segments located at an intercellular boundary.

The outline of cell-wall ingrowth could be clearly discerned from Figs. 2 and 4 in the previous paper⁸⁾. This enabled us to measure the length of plasma membrane increased by the presence of ingrowths. The obtained value was compared with the length of plasma membrane in the case that the existence of ingrowths was ignored, that is, the length of plasma membrane of nucellar cells located at the opposite side. From the result of calculation, the length of plasma membrane traced along the outside of ingrowths was more than three times as long as the plasma membrane estimated at the nucellar cell side. This result is indicative of a higher activity of nutritive transfer by way of cell-wall ingrowth in rice antipodal cells.

The present study and a previous report⁸⁾ show an abundant appearance of ribosomes in rice antipodal cells. From the dense distribution, we could calculate about 7,000 ribosomes in one cubic μm of cytoplasm, because there were about 500 ribosomes in the area of one square μm in the section of $0.07 \mu\text{m}$ thickness. Together with the abundance of rough endoplasmic reticula, this suggests a higher activity of protein synthesis in rice antipodal cells of large size and weak vacuolation.

It has been mentioned that a central cell of

barley is nutritive through the apoplastic route from antipodal cells and nucellar cells⁴⁾. The antipodal cells are mostly functioning until the stage of endosperm cellularization. Therefore, the large size of the antipodal cells and the retention of higher activity in them will lead to large-sized mass of endosperm. This subject seems to be very useful for the achievement of an increase in the grain size of rice.

Based on a cellular morphology of rice antipodal cells before and after anthesis, it is concluded that the antipodal cells have higher activities in energy production and protein synthesis. The active protein synthesis is probably related to the transformation of endoplasmic reticula. And also at the early period of seed formation indeed, the antipodal cells are well able to regulate the flow of nutrients from nucellar tissue into endosperm through functions supported by the morphological characteristics.

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