SOME NEW ASPECTS OF THE PEA (*PISUM SATIVUM* L.) ROOT NODULE ULTRASTRUCTURE

WOJCIECH BORUCKI

Department of Botany, Faculty of Agriculture,
Warsaw Agricultural University,
Rakowiecka 26/30, 02-528 Warsaw, Poland

(Received: November 27, 1995. Accepted: June 15, 1996)

ABSTRACT

Unequal cell divisions were observed in the meristem of pea root nodule. Since after such divisions only the bigger cells become infected then those divisions play a significant role in the formation of the three-dimensional structure of the bacteroidal tissue.

In the infected cells of the young ineffective bacteroidal tissue the first host reaction to the incompatibility of the symbiotic system is the RER membranes aggregation.

In effective symbiosis RER membranes form permanent sites of contact with the peribacteroidal membranes thus connecting all the symbiosoms in the cell. Possibly that ensures the synchronisation of the differentiation processes of the bacteroids and/or their simultaneous degeneration.

The presence of membraneous structures in the form of rings is a characteristic feature of effective bacteroids. It is postulated that the structures are directly connected with nitrogen assimilation. Structures X and Y which are present in the bacteroids of the effective and ineffective symbiosis may be connected with the adaptation of bacterial cells to lowered oxygen pressure in bacteroidal tissue and their transformation (structures X) into bacteroids.

The presence of the cytoplasm (or cytoplasmic remnants) of the infected cells was observed in the intercellular spaces. It is suggested that it is a way, so far unknown, of the gas diffusion regulation in bacteroidal tissue.

KEY WORDS: nitrogen fixation, pea, root nodule development, bacteroidal tissue, bacteroids.

INTRODUCTION

Bacteria of the genus (*Brady*)*rhizobium* are able to induce the formation of root nodules on leguminous plants. In each nodule there is a meristem which produces cells that differentiate themselves into the bacteroidal tissue or cortex cells. The bacteroidal tissue includes two types of cells, i.e. infected cells, in which bacteria are modified into atmospheric nitrogen-fixing forms called the bacteroids, and uninfected cells. The exception is, for example, the peanut and lupine bacteroidal tissues in which the uninfected cells are not present (Sen et al. 1986, Golinowski et al. 1987).

Uninfected cells probably participate in metabolite exchange between infected cells and the nodule cortex which contains vascular bundles and in case of determinated nodules (soybean and bean) the final stages of ureide biosynthesis take place there (Newcomb and Tandom 1981, Hanks et al. 1983). The fulfilment of that function has its structural base consisting in the following: 1.) each infected cell borders upon, at least, one uninfected cell (Selker and Newcomb 1985) and 2.) uninfected cells do not appear separately but form pathways which constitute a bridge between infected cells and the nodule cortex (Selker 1988). Thus it is evident

that the process of cell infection has to be strictly regulated so that the arrangement of cells in the bacteroidal tissue is not accidental. The mechanisms determining which cell is going to be infected and which will remain bacteria-free are still unknown.

A bacterium released from the infection thread in the process of endocytosis is enclosed with a membrane called the peribacteroidal membrane originating from the membrane surrounding the infection thread (Mellor and Werner 1987). The host cell participates in the peribacteroidal membrane development which manifests itself by the increased number of Golgi bodies and RER membranes (Robertson et al. 1978). There are some data attesting to the peribacteroidal membrane features as being similar to the RER membranes (Verma and Stanley 1985), plasma membrane (Tu 1975), plasma membrane and tonoplast (Verma et al. 1978) and also to the outer bacteroidal membrane (Roth and Stacey 1989). There are some suggestions that the RER and peribacteroidal membranes could be continuous which would explain the similarities between them (Newcomb and McIntyre 1981).

Bacteria that gradually differentiate into bacteroids together with the surrounding peribacteroidal membrane and the, so called, peribacteroidal space form a functional entirety de-

scribed as the symbiosom. At the structural level the differentiation of symbiotic bacteria into bacteroids consists in their growth, pleomorphism, changes in cytoplasm osmophilicity, the appearance or disappearance (depending on the genus of bacteria) of polybetahydroxybutyrate (Hirsch et al. 1983, Werner and Mörschel 1978) and sometimes also on the appearance of the membranous structures in their cytoplasm (Dart and Mercer 1963a,b, 1964, Dixon 1964, 1967, Hirsch et al. 1983). Up till now nobody has succeeded in proving the connection between the presence of those structures with a definite function and in particular their connection with nitrogen fixation.

The proper determination of features characteristic for effective symbiosis can be done by the comparison with ineffective symbiosis. In the present paper that comparison was the basis for searching for structural features differentiating the bacteroidal tissue effective in nitrogen fixation from ineffective at different stages of its development. It made possible the attribution of a certain function to the described structures.

MATERIAL AND METHODS

The preparation of plant material for the research *Plant growing.*

Pea seeds (*Pisum sativum* cv. *Six Week*) were surface sterilized with calcium hypochloride suspension for 10 min, then rinsed a few times with distilled water and then for three days germinated at room temperature on the Petri dishes lined with sterile blotting paper wetted with distilled water. Germinated seeds the roots of which reached the length of 2 cm were transferred to pots (five seeds per pot) of 1 liter volume filled with sterile perlite and inoculated with a proper bacterial strain. The plants grew in the photoperiod of 16h light and 8h dark at the temperature of 20-22°C during the day and 14-16°C at night. The plants were lighted with sodium lamp of the WLS4W type by POLAMP. The plants were watered every three days with nitrogen-free medium according to Fahraeus (1957) in the amount of 100 ml per pot and with distilled water on the remaining days.

Inoculation

Pea plants were inoculated with *Rhizobium leguminosarum* bv. *viciae* strain 250a (effective, wild type – nod⁺ fix⁺) or 1064 (ineffective, nod⁺ fix⁻) which were obtained from the collection of the All Russia Research Institute for Agricultural Microbiology in St. Petersburg. For 72 hours the bacteria were multiplied at room temperature on liquid medium at pH = 7, which contained in 1 liter: 0.5 g K₂HPO₄, 0.3 g MgSO₄, 0.1 g NaCl, 10 g saccharose and 0.5 g yeast extract, shaking all the time. Inoculum comprised 20 ml bacterial suspension per pot.

Collecting and fixing plant material

Material for the investigations was collected between 14 and 73 day after inoculation at 7 day intervals. It comprised segments taking from developing and mature parts of nodule bacteroidal tissue. The collected material was fixed in the fixative according to Karnovsky (1965) at room temperature for 4 hours and post-fixed in 1% OsO₄ at 4°C for 2 hours, dehydrated in the increasing concentrations of ethanol, acetone and propylene dioxide and embedded in Epon 812 (Luft 1961).

Ultrathin sections were contrasted with uranyl acetate and lead citrate and examined with electron microscope JEM 100C.

RESULTS

Plant growth

The seeds inoculation by either effective or ineffective bacterial strain resulted in the development of effective (nitrogen fixing) or ineffective (the lack of nitrogen fixation) symbiosis, respectively. The pea plants in both symbioses started to bloom about 3 weeks after inoculation. In the case of ineffective symbiosis symptoms of nitrogen deficiency were distinct 3-4 weeks after inoculation—all leaves turning yellow especially lower ones. At the same time the plants of effective symbiosis did not show any signs of nitrogen deficiency. 8 weeks after inoculation green plants and maturing pods of effective symbiosis contrasted with the plants of ineffective symbiosis which expressed acute nitrogen deficiency (Fig. 1).

Fig. 1. Pea plants infected with effective (+) or ineffective (-) strain of rhizobia – 9 weeks after inoculation.

The development of the bacteroidal tissue *Nodule meristem*

The infection thread develops in the meristematic zone of a 2 week old ineffective nodule (Figs 2a, 2b). That thread is enclosed by a wall continuous with the plant cell wall with the exception of the branch developing within the cell which is undergoing the cell division. Here the infection thread wall disappears and bacteria adhere directly to the membrane surrounding the infection thread. Cell division is unequal which is proved by the place of cell plate formation. The unwallled region of infection can be found in that part of the mother cell which after the division will belong to the bigger cell.

The unequal cell divisions occur also in meristematic parts of effective nodules (data not shown).

Fig. 2a. Anaphase in meristematic part of ineffective nodule.

Fig. 2b. Magnification of the infection thread from Fig. 2a. Note the localization of cell plate (asterisks) as well as walled (arrow head) and unwalled (double arrow head) regions of the infection thread. Bacteria (b) are close to the membrane (arrow) surrounding the infection thread. ch – chromosomes, G – Golgi body, Ma – infection thread matrix, M – mitochondrion, P – plastid, V – vacuole; bars = 2 μ m

Fig. 3. Ultrastructure of young infected cell – effective bacteroidal tissue.

Fig. 4. Ultrastructure of young infected cell – ineffective bacteroidal tissue.

b – bacteroid, Cw – cell wall, G – Golgi body, IT – infection thread, M – mitochondrion, N – nucleus, P – plastid, pm – peribacteroid membrane, ps – peribacteroid space, rer – rough endoplasmic reticulum; bars = 1 μ m.

Young bacteroidal tissue

In young effective and ineffective bacteroidal tissues an intensive development of RER membranes is observed (Figs 3, 4). Ineffective symbiosis is characterized by the RER membranes aggregations thus forming irregular arragments (Fig. 4). In both symbioses mitochondria and plastids are situated in the whole cytoplasm volume of the infected cell although often they appear next to each other.

Mature symbiosis.

Infected cells of mature ineffective symbiosis have more RER and Golgy membranes than in effective symbiosis (Figs 5, 6). Ineffective symbiosomes are characterized by narrow peribacteroid space and large electron-dense regions of bacteroidal cytoplasm. The bacteroids in mature effective bacteroidal tissue are characterized by increased size and pleomorphism (shape variability) and their cytoplasm has no particularly osmophilic areas (Fig. 5).

Fig. 5. Ultrastructure of mature infected cells – effective bacteroidal tissue. Note numerous membranous structures (arrows) in bacteroids (b).
Fig. 6. Ultrastructure of mature infected cells – ineffective bacteroidal tissue.

Cw – cell wall, e – electron-dense region, IS – intercellular space, M – mitochondrion, phb – polibetahydroxybutyrate, pm – peribacteroidal membrane, ps – peribacteroidal space, rer – rough endoplasmic reticulum, S – starch; bars = 1 μ m.

Except PHB-granules three other kinds of structures can be observed in the bacteroids: ring-like, x, and y.

Membranous structures in the form of ring-like flattened vesicles (cisternae) appear in the cytoplasm of effective bacteroids (Fig. 7). Structures of that type do not appear in the cytoplasm of the ineffective bacteroids.

The cytoplasm of effective and ineffective bacteroids also contains oval structures of about 0.2-0.3 μ m having electron dense cortex and non-homogeneous interior (Y structures)

(Figs 8, 9). Y-like structures are occasionally visible in bacteria within infection threads (data not shown).

The third type of bacteroidal structures are the bodies of light cortex part and electron dense core (X structures) (Figs 7, 8). They are often present in effective bacteroids and only sporadically in ineffective bacteroids.

The first to appear in effective bacteroids are the ring-like structures. They can be observed in the mature bacteroid tissues of the two week old nodules. All the above discussed

Figs 7, 8. Various types of structures in effective bacteroids.

Fig. 9. Various types of structures in ineffective bacteroids.

b – bacteroid, c – ring-like cistern, M – mitochondrion, e – osmophilic region of bacteroidal cytoplasm, ps – peribacteroidal space, X and Y – structures in bacteroidal cytoplasm; bars = 0.2 μ m

structures were most numerous in effective nodules collected in 5th and 6th week after the inoculation.

Structural connections of the rough endoplasmic reticulum and peribacteroidal membrane

In the mature bacteroidal tissue of effective nodules collected three weeks after inoculation or older, one can see that the RER membranes are in contact with the peribacteroidal membrane (Fig. 12). The lumen of RER is filled with osmo-

philic material which resembles the material visible in the peribacteroidal space. Sometimes it can be observed that the bulging peribacteroidal membrane forms(?) vesicle filled with the material. At the same time attention is called to the increased osmophilicity of the peribacteroidal membrane surrounding the forming vesicle as compared to its remaining part (Fig. 13). Vesicles (v) resembling those formed by symbiosomes (surrounded by peribacteroidal membrane) can be found in the host cytoplasm (Fig. 15).

Fig. 10. Degenerative changes of plant cells and bacteroids in effective bacteroidal tissue.

Fig. 11. Degenerative changes of bacteroids in ineffective bacteroidal tissue.

asterisks – periplasmic space, b – bacteroid, Cw – cell wall, im – inner bacteroidal membrane, om – outer bacteroidal membrane, IS – intercellular space, M – mitochondrion, N – nucleus, pm – peribacteroidal membrane, ps – peribacteroidal space; bars = 1 μ m.

The connections of RER with the peribacteroidal membrane are either in the form of points or segments (Fig. 14). There has never been observed a continuity of the RER membrane and the peribacteroidal membrane. One RER membrane can fix to more than one peribacteroidal membrane (Fig. 14). The connections of RER with the peribacteroidal membrane remain visible after the bacteroid degeneration (Fig. 10).

Tubular structures in the peribacteroidal space.

During the period preceding the degeneration of effective bacteroids (mature symbiosis of 4-5 week old effective nodules) tubular structures appear in the peribacteroidal space (Figs 15, 16) which spring up from the bacteroid at the places where both bacteroidal membranes are either difficult to be noted or absent. Those structures are surrounded with three

Fig. 12. The linkage of RER membrane with peribacteroid membrane in effective symbiosis.

Fig. 13. Formation of a vesicle filled with osmophilic material – effective symbiosis.

Fig. 14. The linkage of RER membrane with two peribacteroid membranes – effective symbiosis.

asterisks – periplasmic space, b – bacteroid, G – Golgi body, o – osmophilic material, pm – peribacteroid membrane, ps – peribacteroid space, rer – rough endoplasmic reticulum; bars = 0.1 μm.

layer membrane and material contained in them reminds the cytoplasm of the bacteroid. They are in contact with the region of the peribacteroid space into which the osmophilic material from the inside of the RER membranes was introduced. One can also observe a region with numerous fibrillar structures similar to those which are present in the bacteroid cytoplasm and paracrystalline inclusion (Fig. 16).

Degenerative changes of the cells and bacteroids in the bacteroidal tissue.

Fig. 10 presents fragment of 5 weeks old effective bacteroidal tissue. Degenerative processes are visible in two different infected cells. Those processes are of different character which is expressed by the following: a/in left upper cell the host cytoplasm including RER and mitochondria seems to be

Figs 15, 16. Tubular structures in peribacteroid spaces – effective symbiosis.

b – bacteroid, Cw – cell wall, fm – fibrillar material, IS – intercellular space, M – mitochondrion, o – osmophilic material, P – plastid, pi – paracrystalline inclusion, pm – peribacteroid membrane, ps – peribacteroid space, rer – rough endoplasmic reticulum, t – tubular structures, v – vesicle; bars = 0.5 and 0.1 μm respectively.

undamaged similarly as peribacteroid membranes but bacteroids are subjected to lysis or b/in right upper cell the host cytoplasm including RER and mitochondria are damaged similarly as the peribacteroid membrane but the bacteroids remain unchanged.

Bacteroids of ineffective nodules degenerate within the peribacteroid membrane with well preserved structure of the host cytoplasm in which one can observe the RER membranes, mitochondria and cell nucleus (Fig. 11).

In both symbioses the symptom of the bacteroid degeneration is a significant increase of the size of the periplasmic space which results in the fact that the outer and inner bacteroid membranes are clearly separated. At the time when the bacteroid cytoplasm became totally desintegrated, sometimes it is possible to identify those two membranes (Figs 10, 11).

Figs 17, 18. The intercellular spaces filled with the cytoplasm of the infected cells(?) – effective nodules.
 b – bacteroid, Cw – cell wall, M – mitochondrion, pm – peribacteroidal membrane, rer – rough endoplasmic reticulum, S – starch; bars = 0.5 μm .

Intercellular spaces in the bacteroidal tissue

In mature effective bacteroidal tissue there were observed intercellular spaces the contents of which showed the structure of the infected cell cytoplasm with bacteroids surrounded with the peribacteroidal membrane. Apart from those structures, one could be also recognize mitochondria (Figs 17, 18).

DISCUSSION

The development of bacteroidal tissue Nodule meristem.

The data from literature do not show any possibility of infecting cells at the time of their division (Newcomb 1976, Vance et al. 1982). There were no premises allowing the evaluation to what degree or whether it is at all possible to determine the infection process in such a way that would

allow to release bacteria from infection thread into the cytoplasm of only in some types of cells since no structural differentiation was observed in cells "leaving" the nodule meristematic zone (Newcomb et al. 1985) although, undoubtedly, only some of them will be infected. What is more, the arrangement of the uninfected cells in the bacteroidal tissue allowing the formation of a large surface of contact with infected cells and the continuity of that arrangement (Selker 1988) confirm the opinion that cell infection is a strictly regulated process which takes part in the formation of three-dimensional structure of bacteroidal tissue.

It is possible, that an important role in the cell differentiation already within the nodule meristematic zone is played by unequal cell divisions (Figs 2a, b). Both the location of cell plate and the location of the unwallled region of infection thread clearly suggest that after the unequal division only the bigger cell will be infected.

The investigations by Joshi et al. (1991) also revealed the presence of unequal cell divisions in the meristem of the alfalfa spontaneous nodules. The bigger cells appearing as a result of such division accumulated more starch granules than the smaller cells. So also without rizobia nodule meristem can generate two different kinds of cells mimicked infected and uninfected cells of bacteroidal tissue. From that point of view the character of cell divisions in nodule meristem is independent on presence or absence of symbiotic bacteria.

The unequal divisions are often observed in plants preceding for example the differentiation of the root hair cells (Avers 1963, Czernik and Avers 1964), sieve tubes and companion cells (Lüttge and Pitman 1976) or cells forming the stomatal apparatus (Galatis et al. 1984). Thus in case of nodule the unequal division of the meristematic cell would determine which daughter cell is infected and which becomes the uninfected cell. That way of division would also ensure the proper cell arrangement in the bacteroidal tissue in which according to Selker and Newcomb (1985) each infected cell borders with, at least, one uninfected cell.

To my knowledge the results presenting in this paper are the first report about unequal cell divisions in meristematic part of infected nodules and their possible role in the formation of three-dimensional structure of bacteroidal tissue.

Young symbiosis

The increase of the RER membrane number in the cytoplasm of young infected cells is a well known phenomenon (Robertson et al. 1978, Newcomb and McIntyre 1981). However, in the accessible literature on bacteroidal tissue development there is no information about the possibility of the RER membranes accumulations in bigger aggregations, a fact observed in the present investigation in the ineffective and never observed in the effective symbiosis (Figs 3, 4). The aggregation of RER membranes can be connected with the disturbances in the peribacteroidal membrane development in which that organelle takes part. That phenomenon can be a significant cytological indicator of ineffectivity of the symbiosis at a very early stage of the pea nodule bacteroidal tissue development when it is difficult to distinguish the effective and ineffective symbioses on the basis of the evaluation of the degree of bacteroid differentiation. However, in view of the lack of comparative data in literature it is difficult to talk about the universal character of the discussed phenomenon in the pea ineffective nodules formed with the participation of other *Rhizobium* strains and also in ineffective nodules formed on other plants.

Among others, the RER role in nodules consists in increasing of the surface of the membrane surrounding the infection thread (Vance et al. 1982) and increasing the surface of the peribacteroidal membranes (Hirsch et al. 1983). Thus the fact of increased proliferation of the RER membranes during the period of releasing bacteria from the infection thread is comprehensible. On the other hand, it is more difficult to explain why the number of the RER membranes in ineffective symbiosis exceeds that of effective symbiosis. According to McKenzie and Jordan (1974) the increased proliferation of the RER membranes in ineffective symbioses is connected with severe nitrogen deficiency. However, that explanation is not fully satisfactory in case of young symbiosis of pea nodules collected two weeks after the inoculation when symptoms of nitrogen deficiency are not visible. On the other hand, Hirsch et al. (1983) represent the opinion that the numerous RER membranes and Golgi bodies in the ineffective nodules are the symptoms of abnormal synthesis and export of nodulins.

Mature symbiosis

One of the characteristic features of bacteroid maturation is the variability of its shape (Fig. 5) which is defined as pleomorphism (Vasse et al. 1990). The ineffective bacteroids (Fig. 6) are rather rounded or slightly elongated and do not branch off which proves that they do not mature. That thesis is confirmed by the presence of the polybetahydroxybutyrate (PHB) granules in the ineffective bacteroids and the lack of the ring-like structures (cisternes) within their cytoplasm (Fig. 6). Those cisternes characteristic for the effective bacteroids (Fig. 5), could be the site of processes important for effective symbiosis including nitrogenase activity. It is because in the bacteroid plasmic membrane (inner membrane), which probably forms those structures, there are situated the respiratory enzymes which use oxygen. What is more, it is interesting that the cisternes also appear in the bacteroid cytoplasm of the alfalfa and clover nodules (Dixon 1964, 1967, Hirsch et al. 1983) which are similarly as pea nodules resistant to higher oxygen concentrations than in case of, for example, soybean nodules (Rosendahl and Jakobsen 1988).

Structures Y, probably non-membranous seem to be connected with the "state of symbiosis" because they appear (or become distinct!) in the bacteria which are in the terminal fragments of the infection threads before the bacteria release into the host plant cytoplasm (data not shown). Since they appear in both effective and ineffective nodules, then their presence do not depend on the effectiveness of the symbiosis.

Structures X observed only sporadically in ineffective bacteroids and often in mature effective bacteroids can be connected with their maturation. Both structures can be important for adaptation of bacteroids to lowered oxygen pressure in bacteroidal tissue or other stresses. Unfortunately there are no data allowing the attribution of a strictly defined function to structures X and Y.

Similarly interesting are the sites of contact of the RER membranes and the peribacteroidal membrane (Figs 12, 14) which remain visible even after the bacteroid degradation (Fig. 10). It is not unlikely that they play a role just in the process of the bacteroid degradation although their numerous presence already in the bacteroidal tissue of the three week old effective nodules, i.e. still effectively functioning, suggests that they can be the expression also of other processes. Fig. 14 presenting the contact of the RER membranes with the peribacteroidal membrane of two different symbioses warrants the statement that the spatial analysis would reveal

the presence of a continuous system binding groups or even all the symbiosomes through the RER. Because of the participation of the endoplasmic reticulum in the intracellular transportation (Vitale et al. 1993) it could ensure the synchronisation of processes taking place in the bacteroids connected with the process of nitrogen fixation and/or bacteroids simultaneous degradation. That suggestions are confirmed by the accumulation of the electron dense material, similar to that which fills the RER lumen, within the peribacteroidal space close to the "point-like" contact sites between the RER and peribacteroidal membrane (Fig. 12). The way of postulated here transportation of the material from RER lumen to the peribacteroidal space remains in question as no continuity between RER and peribacteroidal membranes was observed. That material can be also identified in the vesicles probably detaching themselves from the symbiosomes (Figs 13, 15). Similar vesicles were observed by Kijne and Planque (1979) in the pea and soybean nodules but according to them they separate from the RER and then fuse together with the peribacteroidal membrane. Unfortunately the contents of those vesicles has not been examined so far. Kijne and Planque (1979) did not observe the permanent contact of RER and peribacteroidal membranes.

During the period directly preceding the bacteroid degeneration in the peribacteroidal space there appear some tubular structures (Figs 15, 16) which, on one side, are in contact with the bacteroid from which they grow and on the other, with the osmophilic material originating from inside of the RER membranes. It looks in such a way as if some bacteroid cytoplasm was transferred to the peribacteroidal space with the help of tubular structures since, as it can be seen, the fibrillar material in the peribacteroidal space looks like that in the bacteroid cytoplasm (Fig. 16). The sense of this phenomenon is mysterious although it could be assumed that it is the introduction to the bacteroid degeneration.

Degenerative changes of the cells and bacteroids.

Fig. 10 shows two different methods of degeneration of the infected cells of effective nodule. The degeneration of bacteroids within undamaged peribacteroidal membranes is usually observed feature of infected cell starvation.

The degeneration of the host cytoplasm and peribacteroidal membranes with well preserved structure of bacteroids (upper right cell), observed occasionally, could result from the degeneration processes escaping the host control or it could be the artefact resulting from the cell damage at the time of collection or preparing the research material. However, it can not be excluded that bacteroids can dedifferentiate into bacteria growing then saprophytically in degenerating bacteroidal tissue.

Intercellular spaces.

Werner and Mörschel (1978) suggest that the *de novo* formation of peribacteroidal membranes around bacteria present in the infection threads developing in the intercellular spaces of bacteroidal tissue of the soybean nodules is possible. Thus the origin of peribacteroidal membranes from the membrane surrounding the infection thread (Vance et al. 1982) would be only one of the methods of their formation. Figures 17 and 18 show that the infected cell cytoplasm or cytoplasm remnants with its characteristic element such as symbiosomes can be seen in the intercellular spaces. Similar situation can take place in soybean which would explain the presence of the peribacteroidal membranes in the intercellular spaces observed by Werner and Mörschel (1978). The data from literature point to the possibility of the regulation of intercellu-

lar space permeability by filling them with water (Hunt et al. 1988) in which the diffusion of gases becomes a thousand times slower or with glycoproteins similar to those present in the infection thread matrix (James et al. 1991). In turn Witty et al. (1987) present the opinion that the changes of the cell turgor regulate the permeability of the intercellular spaces and at the same time adjusting the nodule to the variable oxygen conditions.

Another method of regulating the oxygen flow to the bacteroidal tissue could consist in occlusion the lumen of the intercellular spaces with fragments(?) or remnants of infected cells. A decrease in turgor of infected cells could allow them to change their shape and occlude intercellular spaces but the lack of cell wall and probably plasma membrane surrounding the fragment of infected cell (Fig. 18) remains unclear. So the phenomenon needs further investigations.

In conclusion, this work documents among others the unequal cell divisions in nodule meristem of pea root nodules that are followed by the infection of bigger cell only, structures X and Y in bacteroids, tubular structures in peribacteroidal space, permanent contact of RER and peribacteroidal membranes, discusses a new way of gas diffusion regulation within bacteroidal tissue. I hope that this study reveals new interesting aspects of nodule structure which prompt to further investigations.

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KILKA NOWYCH ASPEKTÓW ULTRASTRUKTURY BRODAWEK KORZENIOWYCH GROCHU (*PISUM SATIVUM* L.)

STRESZCZENIE

W merystemie brodawek korzeniowych grochu zaobserwowano nierówne podziały komórkowe, po których tylko większe komórki są infekowane a mniejsze pozostają niezainfekowane. Podziały te odgrywają istotną rolę w tworzeniu struktury trójwymiarowej tkanki bakteroidalnej, której istotnym elementem jest zapewnienie kontaktu pomiędzy komórkami zainfekowanymi i niezainfekowanymi.

Stwierdzono, że w komórkach młodej nieefektywnej tkanki bakteroidalnej agregacja membran RER jest pierwszą reakcją gospodarza na niekompatybilność układu symbiotycznego.

W symbiozie efektywnej membrany RER tworzą trwałe połączenia z membranami peribakteroidalnymi łącząc w ten sposób wszystkie symbiosomy w komórce, co może zapewniać synchronizację procesów różnicowania się bakteroidów i/lub ich równoczesną degenerację.

Obecność struktur obłonionych w formie pierścieni to charakterystyczna cecha efektywnych bakteroidów. Postuluje się, że te struktury są bezpośrednio związane z wiązaniem azotu. Struktury X i Y obecne w bakteroidach symbiozy efektywnej i nieefektywnej mogą być odpowiedzialne za adaptację komórki bakteryjnej do obniżonego stężenia tlenu w tkance bakteroidalnej i ich transformację (struktury X) w bakteroidy.

W przestworach międzykomórkowych zaobserwowano obecność cytoplazmy komórek zainfekowanych. Sugeruje się, że jest to dotąd nieznan sposób regulacji dyfuzji gazów w tkance bakteroidalnej.

SŁOWA KLUCZOWE: wiązanie azotu, groch, rozwój brodawki korzeniowej, tkanka bakteroidalna, bakteroidy