THE LI-RINGS IN POLLEN TUBES

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ABSTRACT

Monoclonal antibodies, which recognize the specific epitopes for pectins and arabinogalactan proteins, in connection with confocal laser scanning microscopy demonstrated the presence of a ring-like structure in the cell wall of pollen tubes of flowering plants.

KEY WORDS: pollen tube wall, pectins, arabinogalactan proteins, periodic wall structure, LI-rings, stylar structure.

It is nearly impossible to get longitudinal sections of straight pollen tubes over a long segment by the classical techniques of thin-sectioning for electron microscopical observation. Until optical sectioning by confocal laser scanning microscopy (CLSM) allows observations along the length of the pollen tubes. In combination with the application of monoclonal antibodies (MAbs) which recognize specific epitopes, it is now possible to get a more detailed insight of pollen tube wall structure. Of course, pollen tube wall structure is intimately linked to the directed pollen tube growth in the style (Steer and Steer 1989), and the metabolic interaction between tube wall and the surrounding substrates (Rodkiewicz 1973). These pathways can be of slimy nature in hollow styles, or an intercellular matrix in solid styles. The pollen tube wall structure differs in various zones between apex and the germination pore of the pollen grain (Cresti et al. 1980). In general, the pollen tube wall is constructed of three layers, which can be distinguished by their chemical nature: the outer sheath is pectic, the middle layer consists mainly of cellulose microfibrils, and the inner lining is callosic (Heslop-Harrison 1987).

One of the most important discoveries concerning the structure of the pollen tube during the last years, which is linked with the work of Li Yi- Qin and collaborators, is the ring-like pattern in the inner layer and the out sheath of the tube wall (Li et al. 1992a, 1992b, 1994, 1995). These investigators have adapted the MAbs techniques to get insight into the structure of the pollen tube walls.

MAbs MAC 207 and JIM 8 recognize arabinogalactan protein (AGP) epitopes, MAb JIM 5 recognizes polygalacturonic acid epitopes, and MAb JIM 7 recognizes methyl-esterified pectin epitopes. By the use of these MAbs it is possible to distinguish AGPs, unesterified and esterified pectins in the cell wall of pollen tubes. Application of these MAbs in connection with CLSM to *in vitro* grown pollen tubes displayed a periodic distribution of the labelled epitopes along the cell wall of the tubes, which was first observed in *Nicotiana tabacum* (Fig. 1). The ring-like periodic structure

of AGPs and pectins, which we like to call as "LI-ring", have a diameter of approximately 12 μm , the distance between two rings is about 4-7 μm . The correspondence of the diameters of the ring and the pollen tube was found in all species which display LI-rings, so that a genetic background can be concluded.

Optical sectioning by CLSM (Fig. 2) confirms that the LI-rings are present also in the cell wall of pollen tubes produced by the semi *in vivo* technique.

The presence of ring-like pattern of unesterified pectin deposition in the wall of pollen tubes could be detected not in all species investigated, but in some species, such as *Canna*, *Zea*, *Jasminum*, *Cestrum*, *Petunia*, *Solanum*. These species all have a style with a solid transmitting tissue, wherein the pollen tubes have to grow in the intercellular matrix among the cells. On the contrary, in some species which have an open stylar canal, like *Pancratium*, *Saintpaulia*, *Coronilla*, *Hycinthus*, *Lachenalia*, *Lilium*, *Scilla*, *Tulipa*, *Prunus*, no ring-like structure in the cell wall of pollen tubes observed (Li et al. 1994). So one can hypothesize that the LI-rings of the pectin sheath have a function related to the pollen tube growth in the respective styles. The ring-like structure may reinforce the pollen tube wall for its passing through the transmitting tissue. The periodic deposition of unesterified pectin in *Nicotiana tabacum* pollen tube wall has further been confirmed by immunogold labelling with MAb JIM 5 (Geitmann et al. 1995). Furthermore, the co-localization of AGP epitopes and pectin epitopes in pollen grain wall and pollen tube wall has been also demonstrated by immunogold labelling with these antibodies (Li et al. 1995).

It is a well known fact that pollen tubes are subjected to the osmotic pressure in the surrounding cells of the transmitting tissue. In cross sections of styles after self pollination, when the relationship between pollen tubes and style is interrupted, collapsed and squeezed tubes are found frequently (Linskens and van der Pluijm 1966, van der Pluijm and Linskens 1966, Fig. 2). Esterified pectin, which is not aggregated by Ca^{2+} cross-links, was located predominantly at the apex where the

Fig. 1 *Nicotiana tabacum* pollen tubes grown in BK medium (Brewbaker and Kwack 1963) and labelled with MAb MAC 207 to localize AGPs. The periodic rings appeared along in the cell wall along the tube length except the apex. The image was taken by light microscopy (Zeiss, Oberkochen, FRG) Bar: 25 μ m.

Fig. 2 Confocal laser scanning microscopy of a pollen tube of *Nicotiana tabacum*. The pollen tube was grown out of the cut end of the upper style (semi *in vivo* technique) and labelled with MAb JIM 8 to localize AGPs in the cell wall. The picture represents 16 X-Y optical sections (from left-top to the right-bottom) of a part of the pollen tube, which were taken at 0.5 μ m distance in the Z-direction. For each optical section the fluorescence image shows the distribution of the AGP epitopes (upper) and the transmission light image shows the corresponding pollen tube wall (lower). Bar: 25 μ m.

Fig. 3 Confocal laser scanning microscopy of pollen tubes labelled with MAb JIM 5 to localize unesterified pectin in the cell wall; a) *Nicotiana tabacum* pollen tube grown in BK medium (Brewbaker and Kwack 1963); b) *Petunia hybrida* pollen tube grown *in vivo*. The pollen tube was isolated from the transmitting tissue of the style 16 h after pollination. Bars: 25 μ m.

Fig. 4 Confocal laser scanning microscopy of pollen tubes of *Petunia hybrida* grown in a PEG medium (Zhang and Croes, 1981) for 24 h and labelled with MAb JIM 5. Regularly distributed rings were shown in the cell wall of long pollen tubes with length ranging from 2.4 mm to 4.1 mm. Bar: 50 μ m.

pectin esterification provides "loosening" of the cellulose matrix in the wall. This is required for the extension of the tube in the tip region (Muhlethaler and Linskens 1956, Sassen 1964). Deesterification leads to a more rigid structure, so that the pollen tube walls can resist the osmotic pressure of the surrounding cells. The LI-rings are elements of the special construction of the pollen tube walls. Consequently the tube has not to built up a higher osmotic value to resist that of the encircling tissue.

Based on the evidence that the periodic annular distribution of pectin in pollen tube wall is only found in species which have solid styles among 30 species ranging over various levels of plant phylogeny, one may speculate that stylar evolution might be related to the architecture of the pollen tube walls. If this is true, it would be, at the same time, a nice example of co-evolution between sporophyte and gametophyte in Angiosperms.

Finally, it has to be mentioned that the formation of LI-rings is not restricted to pollen tubes grown *in vitro*, which is sometimes a non-optimal condition for growth and metabolism. LI-rings were also observed in pollen tubes grown *in vivo* which had been isolated after passage through the transmitting tissue (Fig. 3). The labelling pattern in the outer surface of the tubes grown *in vivo* differs from the one of the tubes grown *in vitro* by showing spine-like spikes. This could be the result of the intensive interaction between the extracellular enzymes and their substrates located in the pollen tube wall or related to the dissolution of the pectic layer in the pollen tube pathway (Van Amstel 1994).

Apparently the LI-rings have another function than the regularly deposited callose plugs which are present in most types of pollen tubes, either grown *in vitro* or *in vivo*. The callose plugs are found in increasing numbers after the incompatibility reaction which may result from the disturbed metabolic pathway (Linskens and Esser 1957, Tupy 1959, Schlosser 1961). Whereas, the presence of LI-rings in pollen tube wall seems related to the pollen tube growth manner under a certain condition. A pulsatory growth pattern displayed by *Petunia* pollen tubes seems corresponded to the periodic deposition of pectin in the cell wall of pollen tubes (Pierson et al. 1995). The spectacular regularity of the rings distributed along the pollen tube length (Fig. 4) predicts that a precise control mechanism of synthesis and transport of the precursors to form new plasma membrane and cell wall could be discovered by the further study.

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PIERŚCIEŃ-LI W ŁAGIEWKACH PYŁKOWYCH

STRESZCZENIE

Przeciwciała monoklonalne, które rozpoznają specyficzne epitopy pektyn i białek arabinogalaktanowych wiążą się ze ścianą rosnących łagiewek pyłkowych roślin kwiatowych. W mikroskopie konfokalnym na ścianach łagiewek pyłkowych rosnących *in vitro* wykryto obecność pierścieniowatych struktur zawierających te komponenty.

SŁOWA KLUCZOWE: ściana łagiewki pyłkowej, pektyny, białka arabinogalaktynowe, periodyczna struktura ściany, pierścień LI, struktura szyjki słupka.