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THE ROLE OF CELL WALL IN PLANT EMBRYOGENESIS

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Abstract: This review presents recent data about cell wall involvement in plant embryogenesis. During plant development, the cell wall is subjected to precise regulation. During this process a bidirectional information exchange between the cell wall and the protoplast is observed. The cell wall also mediates in the cell-cell (apoplastic) and cell to cell (symplastic) information flow. Especially some products derived from the hydrolysis of specific cell wall compounds can act as short distance signal transduction molecules during the development. Oligosaccharins are a group of such products. Their activity and sources focused the researchers' attention on the biochemical composition of the cell wall and the activity of some cell wall enzymes. The dramatic influence on the embryo body shape has also the cell wall synthesis machinery, including vesicular secretion pathways. Moreover, the interplay between the turgor pressure and counteracting cell walls and neighbouring cells (in higher organisms) creates the specific mechanical forces influencing the development of the whole plant. We conclude that discovering factors which can influence cell wall physiology and architecture is crucial for a better understanding of plant embryogenesis. In this review we summarize some recent experimental data reporting plant cell wall involvement in embryogenesis, putting special emphasis on somatic embryogenesis.

Key Words: Cell Wall, Embryogenesis, Oligosaccharins, Cell Wall Signalling

INTRODUCTION

In recent years the cell wall has been the subject of extensive research also because of the role it plays in many developmental processes. The modern definition describes it as a specialized type of extracellular matrix capable of

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withstanding intracellular hydrostatic pressure [1]. Changes of the chemical composition of the cell wall during plant development alter the cell's physical properties. These changes are correlated in a significant way with the basic functions fulfilled by the wall, i.e. regulation of cell growth, strengthening of tissues, or protection against attack by microorganisms [2, 3]. Although different functions are fulfilled by only two types of the primary cell wall, there are many cell types surrounded by specialized cell walls. It can thus be expected that the results of biochemical analyses of cell wall components do not always reflect the functions it fulfils. Therefore, the function of the cell wall should be considered at the level of complex structures such as tissues, organs and whole organisms. This point of view is in agreement with the organismal theory stating that not only cell lineage determines the overall plant pattern, but also the neighbouring cells play a crucial role [4]. The positional information can be exchanged between neighbouring cells via the symplast and apoplast. Recently the plasmodesmata pathway of information flow rapidly gains importance among the mechanisms of plant development. It seems that plasmodesmata distribution is under tight genetic control, but the mechanism remains largely unknown. There are known non-cell-autonomous proteins that take part in embryogenesis, but regulation of their diffusion through plasmodesmata is still hypothetical [5]. The cell wall is not only the place but also an active component of signal transduction. For example, during *Fucus* embryogenesis deposition of sulphated polysaccharides secreted by the zygote was found as the marker for cell polarity determination [6, 7].

Cell wall-derived substances participate during early stages of higher plant embryo development when an intensive exchange of signals between the cells takes place (Fig. 1). As a result of successive divisions, three basic domains of the embryo differentiate: apical, central and basal. The determination of cell fate in the period of embryonic development is reflected in further development of the plant. The aim of this article is to present information about the role of the cell wall and its changes during embryogenesis.

EMBRYOGENESIS AS A MODEL IN INVESTIGATING THE MOLECULAR BASIS OF DEVELOPMENTAL PROCESSES

Embryos are a convenient object for the observation of events linked to formation of the cell wall, its role in signal perception and the establishment of the primary shape of the plant. During embryogenesis basic plant structure patterns are established and its individual functional domains are determined. In recent years a number of experimental data have been detected concerning the genetic regulation of embryogenesis in plants [8]. The influence of mechanical properties of the cell wall on embryo patterning was also considered [9].

The possibility of the induction of embryo formation from somatic cells facilitates the observation of events occurring during embryogenesis at the cellular level. Specific culture conditions induce biochemical and structural

changes in the cells, among others the loss of traits linked to earlier states of tissue specialisation. Somatic embryogenesis has been precisely characterised in carrot (*Daucus carota*) cell suspension cultures in which under the influence of auxin undifferentiated cells form a proembryogenic mass (PEM) [10]. The transfer of proembryogenic mass to an auxin-free medium causes the formation of somatic embryos [10]. The question whether zygotic and somatic embryogenesis is under the same regulatory mechanism is still to be answered, but the fact is that many similarities were found [11, 12].

The fates of progeny cells formed as a result of mitotic division in an embryogenic culture are different: from one a somatic embryo is formed, whereas the other partly reforms the proembryogenic mass by further cell division. To some extent this resembles the pattern of differentiation of the embryo and the suspensor during zygotic embryogenesis. The two cells formed as a result of the first division are analogues of the basal and apical cells [13].

In the early stages of somatic embryogenesis the spatial organisation of the embryo is regulated to a large extent by positional information. In recent years many experiments have been performed in order to determine the character of the transmitted signals. The results of these investigations indicate that the cell wall participates in the control of growth and differentiation of cells during embryogenesis in plants [14].

CHEMICAL PROPERTIES OF THE CELL WALL AND EMBRYOGENESIS

Synthesis, activity and degradation of different components in the apoplast can be precisely regulated during the course of morphogenesis, leading to numerous changes in physicochemical properties of the cell wall. Many compounds, loosely or strongly bound to the cell wall, participate in regulation of embryogenesis. The regulation itself is complex, depends on gene expression, transport, and enzymatic activity of individual molecules often involved in the intra- and extracellular signal transduction.

Chitinases and arabinogalactan proteins

Much information on substances which could play a potential role in intercellular signal transduction has been provided by investigations performed on a temperature sensitive carrot cell line *ts11*. The *ts11* line obtained by chemical mutagenesis [15] is characterised by the arrest of embryo development at the globular stage at 32°C. Subsequent embryo development is rescued after adding a mixture of proteins secreted by wild-type embryogenic cells to the *ts11* culture [16]. One of those proteins is a glycosylated acid endochitinase EP3 with a molecular weight of 32 kD [17]. The presence of EP3 in the medium was strictly correlated with reestablishment of the ability of *ts11* cells to pass from the globular to the heart-shaped stage. Further experiments showed that *ts11* phenotype had an impaired mechanism of chitinase secretion [18]. At that time

components of the cell wall, which could be used as a substrate by the EP3 endochitinase, were not known (oligomers of at least three N-acetylglucosamines). If such a substrate exists, the product of the reaction catalysed by EP3 could be a compound analogous to the nodulation factor (Nod), a signal molecule produced by bacteria from the genus *Rhizobium* [19]. De Jong *et al.* [20] proposed a mechanism in which endochitinase would participate in the formation of signal particles with a function analogous to Nod factors from a specific precursor molecules having N-acetylated glucosamine residues present in a low amount in the plant cell wall. In order to prove this, many components containing N-acetylglucosamines were tested with respect to restoration of embryogenic potential. In this way they found that the lipooligosaccharide NodRlv-V (Ac, C18:4) from *Rhizobium leguminosarum bv viciae* was as effective as the EP3 protein.

The correlation of chitinase secretion in a *Picea abies in vitro* culture with the ability of PEM to form normal somatic embryos was also demonstrated [21]. The evidence concerning a potential role of chitinases in short distance signal transduction also comes from investigations of the *elp1* mutation in *Arabidopsis*. The *elp1* mutant is characterised by abnormal embryo development caused by ectopic lignin deposition [22]. The *AtCTL1* gene was isolated and its constitutive expression was shown to suppress the *elp1* phenotype [23]. The *AtCTL1* gene encodes a protein with a structure similar to that of chitinases and is not induced by stress. This may suggest that, in contrast to many known chitinases, the activity of this protein is not related to a pathogenesis process. The authors postulate that developmental defects observed in the *elp1* mutant can be related to the lack of activity of the chitinase-like protein taking part in cleaving oligosaccharide fragments playing the role of signal molecules. But till this time there have been no chitin or chitosan type polymers detected in higher plant cell walls, and that is why the next and critical step to prove this hypothesis is to find a substrate for chitinases. In some reports researchers suggest that a special class of arabinogalactan (AGPs) proteins containing N-acetylglucosamine can serve as such a substrate. Since AGPs are soluble and diffusible components of the ECM and the plasmalemma, they are good candidates to act as cell positional markers or as messengers in cell-cell interactions during differentiation [24]. The use of monoclonal antibodies binding appropriate epitopes of arabinogalactan has made it possible to analyse the occurrence of arabinogalactan proteins (AGPs) localised in membranes, cell walls, and secreted to the medium, during embryo formation. Investigations have shown that the JIM8 antibody binds the cell wall of spherical and vacuolised cells (stage B) and does not bind spherical, rich in cytoplasm cells of stage C [25]. In order to label and subsequently isolate single cells from carrot cell suspensions the authors used JIM8 antibodies linked to paramagnetic beads. The authors proved that C type cells are able to form somatic embryos only in the presence of B type cells binding the JIM8 antibody. On this basis a hypothesis was formulated that using JIM8 antibody binding

special AGPs epitopes in plant cell walls it is possible to identify cells involved in cell-cell communication.

The role of arabinogalactan in the transfer of intercellular signals can be also revealed using the Yariv's reagent, which binds to arabinogalactan proteins [26]. The addition of this synthetic phenylglucoside to the medium blocks somatic embryogenesis, which was shown for example in suspension culture of chicory cells [27].

In order to investigate the correlation between the wall bound AGPs and somatic embryogenesis in liquid carrot cell culture Van Hengel *et al.* [28] removed enzymatically the cell wall. The obtained protoplasts had a decreased ability to form somatic embryos. The authors showed that addition of isolated extracellular AGPs partially reversed the effect of cell wall removal. The strongest rescue effect was observed when chitinase-treated AGPs were added. The positive effect of AGPs for embryo formation from protoplasts was observed only before cell wall formation. Moreover, the authors experimentally showed that AGPs secreted into the culture medium and localised in immature seeds contained glucosamine and N-acetyl-D-glucosaminyl. On this basis the authors suggest that some AGPs can be a substrate for chitinases. It was also shown that AGPs could reinitiate cell divisions in non-dividing protoplasts.

The ability of chitinase-like proteins (CLPs) to cleave the sugar chains of arabinogalactan proteins has also been shown in embryogenic cell lines of the pine [29] and carrot [30].

The experiments described above suggest an essential role of chitinases and cell wall AGPs in plant embryogenesis. Recently Dyachok *et al.* [31] found that there is possibly some other than AGPs substrate for chitinase in the plant cell wall. A chitooligosaccharide stimulating the cell divisions in proembryogenic mass was isolated from embryogenic cultures of *Picea abies*. Similarly to the Nod factors of *Rhizobium*, it was found to be a lipophilic compound containing N-acetylated glucosamine residues and being a substrate for chitinases. The addition of chitinase, the Nod factor, or a partly purified culture filtrate containing chitooligosaccharides substituted for the presence of synthetic auxin 2,4-D in the culture. The authors suggest that chitooligosaccharides, similarly to 2,4-D, stimulate the early stages of somatic embryogenesis by the induction of cell divisions and inhibition of the cell death in PEM [31]. It seems, however, that different chitinases may regulate embryogenesis in different ways. While the EP3 chitinase in carrot and the *Streptomyces griseus* chitinase in spruce induced the formation of lipophilic chitooligosaccharides (LCOs) [28, 31], a chitinase with a molecular mass of 28 kD secreted by somatic spruce cells (type A) decomposed chitooligosaccharides [31]. A similar phenomenon exists in the case of chitinase dependent degradation of Nod factors [32].

Summarising, it can be stated that chitinases, through participation in production or degradation of chitooligosaccharides, may play a regulatory role in embryogenesis. The products of chitinase activity are oligosaccharins [33] playing important role in somatic embryogenesis. To describe the signalling

pathways involved in these responses more analyses are required. A schematic representation of the action of chitinases and products of cell wall degradation in the dynamic cell information flow machinery is shown in Fig. 1.

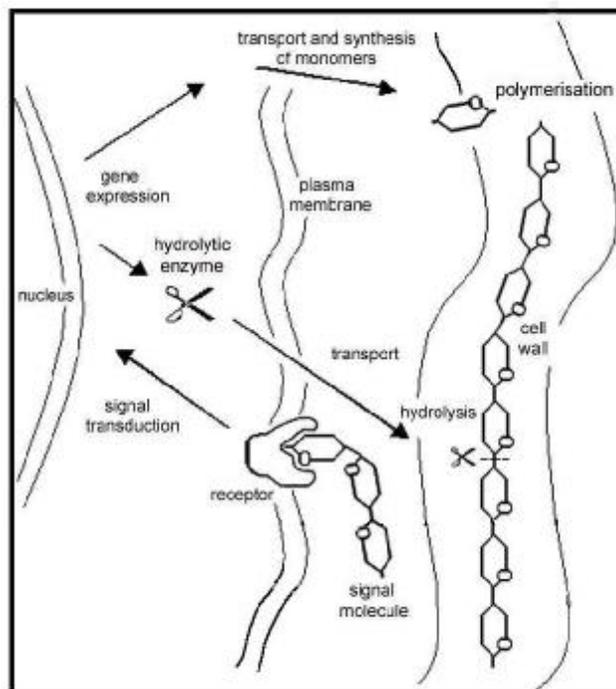


Fig. 1. The mechanism of synthesis, transport and perception of short distance oligosaccharide signal molecules. This mechanism is functional both within a single cell and between neighbouring cells.

The participation of PR proteins in embryogenesis

The cell wall proteins whose induction is related to pathogenesis participate in many developmental processes [34]. This suggests that the mechanism of environmental signal transduction in plants (stresses, microorganism attacks) is used for intercellular developmental signalling.

It was noted that besides two different endochitinases an osmotin, which is a PR-protein, also had an effect on the induction of somatic embryogenesis in a chicory cell suspension [35]. In other experiments the number of chicory somatic embryos formed was positively correlated with an increased amount of β -1,3-glucanase (37.7 kDa). This enzyme participates in the enzymatic cleavage of callose in the cell wall of embryogenic cells and young embryos [36].

Pectins

Pectins and hemicelluloses belong to the major polysaccharide complex of the cell wall. Their synthesis takes place in the Golgi system and they are transported to the cell wall by means of secretory vesicles [37]. The properties of cell walls change during growth and development. In order to ensure an appropriate shape of the cell, these components must be delivered to specific sites in the cell wall. Therefore, secretion of the cell wall components should have a significant effect on plant development. This was demonstrated by investigations on the *Arabidopsis emb30* mutant (also called *gnom*), showing abnormal formation of an apical-basal embryo axis [38]. The product of the *EMB 30* gene was shown to be involved in the transport of pectins to the cell wall via vesicles of the Golgi apparatus. Transport inhibition of pectins, which are the main components of middle lamella, caused impairment of intercellular adhesion resulting in relaxation of the tissue and morphological embryo defects [39]. The role of pectins in cell adhesion during development was also shown in experiments with *quasimodo* (*qual-1* and *qual-2*) mutants of *Arabidopsis* [40]. The corresponding gene *QUAI* encodes a putative membrane-bound glycosyltransferase of family 8, possibly involved in pectin biosynthesis. *Quasimodo* mutants are characterised by abnormal cell shape, have dwarf phenotype and defects in seed formation. The authors suggest that the observed defects are a consequence of weaker adhesion and changed cell wall strength.

The role of pectins in signalling processes during development was also reported. Homogalacturonates, which are pectin polymers, were shown to participate in intercellular signal transduction [41]. Similarly to chitoooligosaccharides, oligogalacturonide molecules released from longer chains participate in processes related to growth and development as well as in resistance responses of the plants [42]. Oligogalacturonides trigger calmodulin-dependent mobilisation of cytosolic free Ca^{2+} , acidification of the cytosol [43], potassium efflux [44] and hydrogen peroxide production [45]. This pectic signal transduction can be additionally modulated by polyamines [43].

Xyloglucan endotransglycosylases – a structural and regulatory function

Xyloglucan transglycosylases (XET) are considered to be a very important factor actively changing the mechanical properties of the cell wall responsible for cell growth [46]. These enzymes increase the plasticity and elasticity of cell walls, due to the ability to cleave xyloglucan chains and insert them at a different site [47]. Many XETs also catalyse the linkage of new xyloglucan oligosaccharides to already existing chains [48, 49].

The XET activity often depends on developmental and environmental factors. Within the gene family coding for xyloglucan endotransglycosylases in *Arabidopsis thaliana*, TCH4 has been identified [50], whose expression is regulated by such factors as: mechanical stimuli, temperature changes, light and growth regulators. The TCH4 protein acts at a pH= 6.0-6.5 and its activity is not linked to the acid growth of the cell. The *Arabidopsis* plants exposed to the wind

shared an increased level of the TCH4 mRNA transcript in the parenchymatic tissue and in the epidermis [51]. These results indicate that the TCH4 protein may contribute to morphogenetic changes appearing as a result of the action of mechanic stimuli. It can be assumed that in flasks and bioreactors used for *in vitro* embryo cultures the mechanical stimuli activation of many genes may occur, leading to unexpected effects [52].

Experiments using antibodies have indicated that xyloglucan endotransglycosylases are accumulated in growing cells in the sites of intercellular air space formation, at the leaf base, and in cotyledons and hypocotyls of a variety of plants (*Arabidopsis*, *Nicotiana*, *Zinnia*, etc.). XET accumulation has also been found in vascular tissue during the formation of tracheary elements and xylem bundles [53].

Xyloglucan endotransglycosylases also act during induction of somatic embryogenesis and in the early stages of embryo development of a cell suspension culture of cucumber (*Cucumis sativus*) [54, 55]. Moreover, it has been proven that from xyloglucans oligosaccharide chains are detached which could participate in transduction of intercellular signals [41, 56].

Peroxidases

Peroxidases take part in processes occurring inside and outside the cell. Their activity is regulated developmentally by changes in the level of calcium Ca^{2+} ions [57]. Some peroxidases have been shown to suppress the inhibition of somatic embryogenesis caused by the addition of tunicamycin to the medium [58]. This substance blocks N-glycosylation and glycosylated peroxidases may decrease its activity. The addition of tunicamycin to the medium induces PEM cell growth and vacuolisation. Peroxidases reduce the plasticity of the cell wall and inhibit further cell growth. The effect of their activity is similar to the removal of auxin from the medium in an embryogenic culture. Moreover, it has been noted that basic peroxidases participate in IAA decomposition inducing the formation of somatic embryos [59].

Peroxidase activity may vary at different stages of the embryogenic suspension culture [60]. Such a situation was observed for soluble peroxidase derived from the walls of embryogenic carrot cells. This difference was threefold in favour of non-embryogenic cells [61]. The higher peroxidase activity may be connected with the stabilisation of an elongated shape of non-embryogenic cells. In the same model distribution of membrane bound peroxidases was different. The embryogenic cells contained a higher level of membrane bound peroxidase compared to the non-embryogenic cells. The main activity of membrane bound peroxidases was detected in secretory vesicles of the intercellular matrix in embryogenic cells. The authors suggest that this may be related to enhanced packaging of peroxidase in secretory vesicles in embryogenic cells.

A precise evaluation of the role of peroxidases in embryogenesis requires further investigations on the location and activity of individual isoenzymes.

MECHANICAL PROPERTIES OF THE CELL WALL

Resistance of the cell wall to mechanical deformation determines its participation in the formation of intracellular pressure. Stimuli derived from changes in tensions of the cell wall trigger a cascade of signals leading to specific cell response. The orientation of cell divisions and the fate of cells during their differentiation can be changed by directly applied force [63]. Moreover, the influence of mechanical stimuli on the embryo patterning was also shown. The first *Fucus* zygote division determines the establishment of the basic embryo polarity. In higher plants, a strong influence of maternal tissue surrounding the zygote and embryo is observed. That is why the plane of the first zygote division not always determines the embryonic axis [10].

The correlation between the cell wall and divisions is very strong. Recently it was found that mutations in the *Arabidopsis thaliana* *RSH* gene encoding an extensin-type cell wall protein cause improper positioning of the cell plate. Probably because of disturbance in cytokinesis, *rsh/rsh* mutants have abnormal embryo development. For the time being we are not able to answer the question whether the RSH protein fulfils a direct function in positioning the cell plate or whether the effect is due to its structural role in cell wall strengthening [64]. There is also an earlier report about the HRGP protein encoding gene involvement in maize embryo cell divisions and differentiation [65]. Involvement of other class structural cell wall proteins was also reported. Differential transcription of the *Atgrp-5* gene encoding the GRP protein has been found during early stages of *Arabidopsis thaliana* somatic embryogenesis [66]. The expression pattern of this gene indicates that presence of this GRP protein is necessary for the first anatomical modifications leading to somatic embryo development. The authors suggest that the AtGRP-5 protein is directly and indirectly involved in mechanical and chemical cell wall modifications necessary to undergo a somatic embryogenic program. To shed more light on mechanical vs. molecular correlation during different stages of embryogenesis, further analyses should be made.

CONCLUDING COMMENTS

Cell signalling is a key factor of many processes including embryogenesis. So far it is difficult to specify exactly what role is played by signal particles cleaved out of proteins or polysaccharides of the cell wall. Oligosaccharins cleaved from polymers of β -1,4-linked N-acetylglucosamine, pectins or xyloglucans fulfil numerous functions in the cell and many of them are awaiting for more detailed analyses. Such biochemical activities of the cell wall overlap with its mechanical properties.

The cell wall participates in embryogenesis by involvement in signal transduction and the formation of tensions influencing the cell shape and division plane. During embryogenesis specific rebuilding of the cell wall takes

place. Cell wall changes can be monitored by observation of differential gene expression, changes in enzymatic activity of individual apoplastic proteins, and different growth and division activities of the cells. A precise knowledge on the embryogenesis regulation and the participation of the cell wall in this process requires an application of many different techniques. Sequencing of whole genomes of model organisms together with a simultaneous investigation of the expression of thousands of genes illustrates the complicated interdependence of many genetical factors involved. The adaptation of methods determining protein interactions to plant systems will allow a better understanding of the cell wall involvement in embryogenesis. The investigations of the whole proteome and metabolome should define the principles of plant cell wall functioning.

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REFERENCES

1. Peters, W.S., Hagemann, W. and Tomos, D.A. What makes plants different? Principles of extracellular matrix function in 'soft' plant tissues. **Comp. Biochem. Physiol.** Part A 125 (2000) 151-167.
2. Sakurai, N. Cell wall functions in growth and development. A physical and chemical point of view. **Bot. Mag. Tokyo** 104 (1991) 235-251.
3. Wojtaszek, P. Genes and plant cell walls: a difficult relationship. **Biol. Rev.** 75 (2000) 437-475.
4. Kaplan, D.R. and Hagemann, W. The relationship of cell and organism in vascular plants - are cells the building blocks of plant form? **Bio Sci.** 41 (1991) 693-703.
5. Haywood, V., Kragler, F. and Lucas, W.J. Plasmodesmata: Pathways for protein and ribonucleoprotein signalling. **Plant Cell Supplement** (2002) 303-325.
6. Quatrano, R.S. and Shaw, S.L. Role of the cell wall in the determination of cell polarity and the plane of cell division in *Fucus* embryos. **Trends Plant Sci.** 2 (1997) 15-21.
7. Corellou, F., Potin, P., Brownlee, C., Kloareg, B. and Bouget F-Y. Inhibition of the establishment of zygotic polarity by protein tyrosine kinase inhibitors leads to an alteration of embryo pattern in *Fucus*. **Dev. Biol.** 219 (2000) 165-182.
8. Grabowska, A., Filipecki, M. and Linkiewicz, A. Genetic regulation of plant embryogenesis. **Adv. Cell. Biol.** 28 (2001) 509-527.
9. Kaplan, D.R. and Cooke, T.J. Fundamental concepts in the embryogenesis of dicotyledons: a morphological interpretation of embryo mutants. **Plant Cell** 9 (1997) 1903-1919.

10. Krikorian, A.D. and Smith, D.L. Somatic embryogenesis in carrot (*Daucus carota*). Lindsey K.. **Plant Tissue Culture Manual**. Dordrecht: Kluwer Academic Publishers, A9 (1992) 1-32.
11. Dodeman, V.L., Ducreux, G. and Kreis, M. Zygotic embryogenesis versus somatic embryogenesis. **J. Exp. Bot.** 48 (1997) 1493–1509.
12. Hecht, V., Velle-Calzada, J.-P., V. Hartog, M., Schmid, E.D.L., Boutilier K., Grossniklaus, U. and DeVries, S.C. The *Arabidopsis* somatic embryogenesis receptor kinase 1 gene is expressed in developing ovules and embryos and enhances embryogenic competence in culture. **Plant Physiol.** 127 (2001) 803-816.
13. Souter, M. and Lindsey, K. Polarity and signalling in plant embryogenesis. **J. Exp. Bot.** 51 (2000) 971-983.
14. Brownlee, C. Role of the extracellular matrix in cell-cell signalling: Paracrine paradigms. **Curr. Opin. Plant Biol.** 5 (2002) 396-401.
15. Giuliano, G., Lo Schiavo, F. and Terzi, M. Isolation and developmental characterization of temperature sensitive carrot cell variants. **Theor. Appl. Genet.** 67 (1984) 179-183.
16. Lo Schiavo, F., Giuliano, G., De Vries, S.C., Genga, A., Bollini, R., Pitto, L., Cozzani, G., Nuti-Ronchi, V. and Terzi, M. A carrot cell variant temperature sensitive for somatic embryogenesis reveals a defect in the glycosylation of extracellular proteins. **Mol. Gen. Genet.** 223 (1990) 4901-4907.
17. De Jong, A.J., Cordewener, J., Lo Schiavo, F., Terzi, M., Vandekerckhove, J., Van Kammen, A. and De Vries, S.C. A carrot somatic embryo mutant is rescued by chitinase. **Plant Cell** 4 (1992) 425-433.
18. De Jong, A.J., Hendriks, T., Meijer, E.A., Penning, M., Lo Schiavo, F., Terzi, M., Van Kammen, A. and De Vries, S.C. Transient reduction in secreted 32kD chitinase prevents somatic embryogenesis in the carrot (*Daucus carota L.*) variant ts11. **Dev. Genet.** 16 (1995) 332-343.
19. Truchet, G., Roche, P., Lerouge, P., Vasse, J., Camut, S., De Billy, F., Prome, J.-C. and Denarie, J. Sulphated lipo-oligosaccharide signals of *Rhizobium meliloti* elicit root nodule organogenesis in alfalfa. **Nature** 351 (1991) 670-673.
20. De Jong, A.J., Heidstra, R., Spaink, H.P., Van Hartog, M., Meijer, E.A., Hendriks, T., Lo Schiavo, F., Terzi, M., Bisseling, T., Van Kammen, A. and De Vries, S.C. *Rhizobium* lipooligosaccharides rescue a carrot somatic embryo mutant. **Plant Cell** 5 (1993) 615-620.
21. Mo, L.H., Egertsdotter, U. and Von Arnold, S. Secretion of specific extracellular proteins by somatic embryos of *Picea abies* is dependent on embryo morphology. **Ann. Bot.** 77 (1996) 143-152.
22. Zhong, R., Ripperger, A. and Ye, Z.-H. Ectopic deposition of lignin in the pith of stems of two *Arabidopsis* mutants. **Plant Physiol.** 123 (2000) 59-69.

23. Zhong, R., Kays, J., Schroeder, B.P. and Ye, Z-H. Mutation of a chitinase-like gene causes ectopic deposition of lignin, aberrant cell shapes, and overproduction of ethylene. **Plant Cell** 14 (2002) 165-179.
24. Fincher, G.B., Stone, B.A. and Clarke, A.E. Arabinogalactan proteins: structure biosynthesis and function. **Annu. Rev. Plant Physiol.** 34 (1983) 47-70.
25. McCabe, P.F., Valentine, T.A., Forsberg, L.S. and Pennell, R.I. Soluble signals from cells identified at the cell wall establish a developmental pathway in carrot. **Plant Cell** 9 (1997) 2225-2241.
26. Yariv, J., Rapport, M.M. and Graf, L. The interaction of glycosides and saccharides with antibody to the corresponding phenylazo glycoside. **Biochem J.** 85 (1962) 383-388.
27. Chapman, A., Blervacq, A.-S., Vasseur, J. and Hilbert, J.-L. Arabinogalactan-proteins in *Cichorium* somatic embryogenesis: effect of β -glucosyl Yariv reagent and epitope localisation during embryo development. **Planta** 211 (2000) 305-314.
28. Van Hengel, A.J., Tadesse, Z., Immerzeel, P., Schols, H., van Kammen, A. and de Vries, S.C. N-Acetylglucosamine and glucosamine-containing arabinogalactan proteins control somatic embryogenesis. **Plant Physiol.** 125 (2001) 1880-1890.
29. Domon, J.-M., Neutelings, G., Roger, D., David, A. and David, H. A basic chitinase-like protein secreted by embryogenic tissues of *Pinus caribaea* acts on arabinogalactan proteins extracted from the same cell lines. **J. Plant Physiol.** 156 (2000) 33-39.
30. Passarinho, P.A., Van Hengel, A.J., Franz, P.F. and De Vries, S.C. Expression pattern of the *Arabidopsis thaliana* AtEP3/AtchiV endochitinase gene. **Planta** 212 (2001) 556-567.
31. Dyachok, J., Wiweger, M., Kenne, L. and Von Arnold, S. Endogenous Nod-factor-like signal molecules promote early somatic embryo development in norway spruce. **Plant Physiol.** 128 (2002) 523-533.
32. Schultze, M. and Kondorosi, Á. What makes nodulation signals host-plant specific? **Trends in Microbiol.** 3 (1995) 370-372.
33. Darvill, A., Augur, C., Bergmann, C., Carlson, R.W., Cheong, J.J., Eberhard, S., Hahn, M.G., Lo, V.M., Marfa, V. and Meyer, B. Oligosaccharins – oligosaccharides that regulate growth, development and defence responses in plants. **Glycobiology** 2/3 (1992) 181-198.
34. Yong, H-Ch., Chang, H-S., Gupta, R., Wang, X., Zhu, T. and Luan, S. Transcriptional profiling reveals novel interactions between wounding, pathogen, abiotic stress, and hormonal responses in *Arabidopsis*. **Plant Physiol.** 129 (2002) 661-677.
35. Hellebood, S., Hendriks, T., Bauw, G., Inze, D., Vasseur, J. and Hilbert, J.-L. Three major somatic embryogenesis related proteins in *Cichorium* identified as PR proteins. **J. Exp. Bot.** 51 (2000) 1189-1200.

36. Helleboid, S., Bauw, G., Belingheri, L., Vasseur, J. and Hilbert, J.L. Extracellular β -1,3-glucanases are induced during early somatic embryogenesis in *Cichorium*. **Planta** 205 (1998) 56-63.
37. Driouich, A., Faye, L. and Staehelin, L.A. The plant Golgi apparatus: A factory for complex polysaccharides and glycoproteins. **Trends Biochem. Sci.** 18 (1993) 210-214.
38. Shevell, D.E., Leu, W.M., Gillmor, C.S., Xia, G., Feldmann, K.A. and Chua, N.H. EMB30 is essential for normal cell division, cell expansion, and cell adhesion in *Arabidopsis* and encodes a protein that has similarity to Sec7. **Cell** 77 (1994) 1051-1062.
39. Shevell, D.E., Kunkel, T. and Chua, N-H. Cell wall alterations in the *Arabidopsis* emb30 mutant. **Plant Cell** 12 (2000) 2047-2059.
40. Bouton, S., Leboeuf, E., Mouille, G., Leydecker, M.-T., Talbotec, J., Granier, F., Lahaye, M., Höfte, H. and Truong, H.-N. QUASIMODO1 Encodes a putative membrane-bound glycosyltransferase required for normal pectin synthesis and cell adhesion in *Arabidopsis*. **Plant Cell** 14 (2002) 2577-2590.
41. Fry, S.C., Adlington, S., Hetherington, P.R. and Aitken, J. Oligosaccharides as signals and substrates in the plant cell wall. **Plant Physiol.** 103 (1993) 1-5.
42. Ridley, B.L., O'Neil, M.A. and Mohnen, D. Pectins: structure, biosynthesis, and oligogalacturonide – related signaling. **Phytochemistry** 57 (2001) 929-967.
43. Messiaen, J. and Van Cutsem, P. Polyamines and pectins. II. Modulation of pectic - signal transduction. **Planta** 208 (1999) 247-256.
44. Mathieu, Y., Kurkdjian, A., Xia, H., Guern, J., Koller, A., Spiro, M., O'Neill, M., Albersheim, P. and Darvill A. Membrane responses induced by oligogalacturonides. **Plant J.** 1 (1991) 333-343.
45. Legendre, L., Rueter, S., Heinstejn, P.F. and Low, P.S. Characterization of the oligogalacturonide-induced oxidative burst in cultured soybean (*Glycine max*) cells. **Plant Physiol.** 102 (1993) 233-240.
46. Vissenberg, K., Verbelen, J-P., Miller, J.G. and Fry, S.C. To expand or not to expand, is Xet the only answer? **Plant Physiol. Biochem.** 38 (2000) 13.
47. Xu, W., Purugganan, M.M., Polisensky, D.H., Antosiewicz, D.M., Fry, S.C. and Braam, J. *Arabidopsis* TCH4, regulated by hormones and the environment, encodes a xyloglucan endotransglycosylase. **Plant Cell** 7 (1995) 1555-1567.
48. Thompson, J.E. and Fry, S.C. Restructuring of wall-bound xyloglucan by transglycosylation in living plant cells. **Plant J.** 26 (2001) 23-34.
49. Sulová, Z., Baran, R. and Farkaš, V. Release of complexed xyloglucan endotransglycosylase (XET) from plant cell walls by transglycosylation reaction with xyloglucan – derived oligosaccharides. **Plant Physiol. Biochem.** 39 (2001) 927 – 932.

50. Purugganan, M.M., Braam, J. and Fry, S. The *Arabidopsis* TCH4 xyloglucan endotransglycosylase. Substrate specificity, pH optimum, and cold tolerance. **Plant Physiol.** 115 (1997) 181-190.
51. Antosiewicz, D.M., Purugganan, M.M., Polisensky, D.H. and Braam, J. Cellular localization of *Arabidopsis* xyloglucan endotransglycosylase-related proteins during development and after wind stimulation. **Plant Physiol.** 115 (1997) 1319-1328.
52. Kieran, P., Malone, D. and MacLoughlin, P. Effects of hydrodynamic and interfacial forces on plant cell suspension systems. **Adv. Biochem. Eng. /Biotechnol.** 67 (2000) 141-177.
53. Campbell, P. and Braam, J. Xyloglucan endotransglycosylases: diversity of genes, enzymes and potential wall-modifying functions. **Trends Plant Sci.** 4 (1999) 361-366.
54. Linkiewicz, A. Isolation and analysis of genes induced and repressed during early stages of somatic embryogenesis of cucumber (*Cucumis sativus* L.). **PhD. thesis**, Warsaw Agricultural University (2001).
55. Kwaśniak, A. Cloning and analysis of genes induced during somatic embryogenesis in cucumber (*Cucumis sativus* L.) cell suspension culture – comparison with the gene expression profile of *Arabidopsis thaliana* Heynh. **PhD. thesis**, Warsaw Agricultural University (2001).
56. Creelman, R.A. and Mullet, J.E. Oligosaccharins, brassinolides, and jasmonates: Nontraditional regulators of plant growth, development, and gene expression. **Plant Cell** 9 (1997) 1211-1223.
57. Penel, C., Sticher, L., Kevers, C., Gaspar, T. and Greppin, H. Calcium-controlled peroxidase secretion by sugar-beet cells: effect of ionophores in relation to organogenesis. **Biochem. Physiol. Pflanz.** 179 (1984) 173-180.
58. Cordewener, J., Booij, H., Van der Zandt, H., Van Engelen, F., Van Kammen, A. and De Vries, S. Tunicamycin – inhibited carrot somatic embryogenesis can be restored by secreted cationic peroxidase isoenzymes. **Planta** 184 (1991) 478-486.
59. Schiavone, F.M. and Cooke, T.J. Unusual patterns of somatic embryogenesis in domesticated carrot: Developmental effects of exogenous auxins and auxin transport inhibitors. **Cell Diff.** 21 (1987) 53-62.
60. Joersbo, M., Andersen, J.M., Okkels, F.T. and Rajagopal, R. Isoperoxidases as markers of somatic embryogenesis in carrot cell suspension cultures. **Physiol. Plant.** 76 (1988) 10-16.
61. Mårtensson, B., Sommarin, M. and Widell, S. Peroxidase activity and ATP – dependent Ca²⁺ transport in subcellular fractions of embryogenic and non – embryogenic *Daucus carota* cell suspensions. **Plant Physiol. Biochem.** 36 (1998) 515-524.
62. Lynch, T.M. and Lintilhac, P.M. Mechanical signals in plant development: A new method for single cell studies. **Dev. Biol.** 18 (1997) 246-256.

63. Hall, Qi and Cannon, M.C. The cell wall hydroxyproline-rich glycoprotein RSH is essential for normal embryo development in *Arabidopsis*. **Plant Cell** 14 (2002) 1161-1172.
64. Sato, S., Toya, T., Kawahara, R., Whittier, R.F., Fukuda, H. and Komamine, A. Isolation of a carrot gene expressed specifically during early-stage somatic embryogenesis. **Plant Mol. Biol.** 28 (1995) 39-46.
65. Ruiz-Avila, L., Burgess, S.R., Stiefel, V., Ludevid, M.D. and Puigdomenech, P. Accumulation of cell wall hydroxyproline-rich glycoprotein mRNA is an early event in maize embryo cell differentiation. **Proc. Natl. Acad. Sci. USA** 89 (1992) 2414-2418.
66. Magioli, C., Barroco, R.M., Benicio-Rocha, C.A., de Santiago-Fernandes, L. D., Mansur, E., Engler, G., Margis-Pinheiro, M. and Sachetto-Martins, G. Somatic embryo formation in *Arabidopsis* and eggplant is associated with expression of a glycine-rich protein gene (*Atgrp-5*). **Plant Sci.** 161 (2001) 559-567.