LECTURES

Induced embryogenesis *via* egg cell activation in cereals

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Embryogenesis is a pivotal stage of the life cycle of flowering plants as it generates the starting material for the next, sporophytic generation. During seed development, embryogenesis is triggered by fusion of the egg cell with one of the sperm cells. The unfertilised egg cell has an inherent competence for embryogenesis, but in general, remains inactive in the absence of fertilisation. Once fertilised, the egg cell becomes activated for embryo development and a series of cellular and sub-cellular events occur that convert the single-celled zygote into a mature embryo. One of the earliest events is the establishment of cellular polarity and a subsequent asymmetric division. The factors that define egg cell competence are poorly understood because the very early events of egg cell activation occur encased in the maternal tissues. In vitro single cell culture systems are a powerful tool for studying early embryogenesis as they provide access to material that is normally difficult to isolate and micromanipulate due to its small size and location within the female tissues.

In the present study, a successful plant regeneration system is presented *via* wheat × rice pollination and subsequent rescue of the activated egg cell. Wheat plants were pollinated with rice and the egg cells were dissected 1–3 DAP and co-cultured with wheat ovaries as previously described (Bakos et al., 2003). Compared to the control, the in vitro development of the egg cells activated by foreign pollination was strongly inhibited during the initial stage, however, many of them became elongated and polarised. This suggest that the cell cycle of these cells were activated but not completed by this time (Kumlehn et al., 1999). Similar features could be detected when cell cycle regulatory genes had been introduced into the unfertilised wheat egg cell by microinjection.

Co-injection of cyclin and cyclin-dependent kinase genes resulted in asymmetric organelle distribution in the cytoplasm and chromatin condensation in the nucleus, which was followed by nuclear division. In the case of foreign pollination only a few activated egg cells were capable to complete the first cell division, but these cells divided further very rapidly and developed in embryo-like structures (ELS). As a final result, four plants from the *Chinese Spring* × *Rigola* and one from the *MvKr1* × *Rigola* crosses has been obtained.

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REFERENCES

BAKOS F, DARKO E, PONYA Z, and BARNABAS B. 2003. Application of wheat (*Triticum aestivum* L.) microspore culture and ovaries to raise wheat zygotes *in vitro. Acta Biologica Cracoviensia Series Botanica* 45: 107–110.

KUMLEHN J, LÖRZ H, and KRANZ E. 1999. Monitoring individual development of isolated wheat zygotes: a novel approach to study early embryogenesis. *Protoplasma* 208: 156–162.

Female gametophyte functions in maize involve pollen tube guidance and discharge

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The female gametophyte (embryo sac) of maize and other cereals consists of the haploid cells of the egg apparatus (egg cell and two synergids), a diploid central cell as well as a cluster of 20–40 antipodals, cells that undergo endoreduplication. All embryo sac cells are derived from the functional megaspore after meiosis. A number of functions have been addressed to the female gametophyte that include pollen tube guidance and discharge, regulation of the double fertilization process, prevention of polyspermy, maternal control of embryo and endosperm development as well as the induction of seed development after fertilization. Until now, most of our knowledge about female gametophyte development and function has been generated through genetic analyses. We are performing an alternative approach and have generated cell-type-specific cDNA libraries from the individual cells of the female gametophyte before and after fertilization. Differential screening of these libraries using methods such as classical plaque screening, SSH, RFDD-PCR, random EST sequencing etc. have been used to identify and clone genes differentially and specifically expressed in the individual cells of the embryo sac. Bioinformatics was applied to select candidate genes that are specifically expressed and whose products might be involved in processes such as cell-cell communication, polarity, gene regulation and cell cycle control.

We will restrict our report today to those candidate genes that encode small proteins, which might be secreted by the embryo sac and that we have selected for functional studies in maize. One gene highly and specifically expressed in the egg apparatus was termed *ZmEA1*. A gene family expressed exclusively in the egg apparatus and central cell was named *ZmES1-4*, a small peptide gene expressed in both male and female gametophytes *ZmTLA1* as well as a small secreted protein whose expression is correlated to cell division *ZmEC222*. Functional studies with ZmEA1 revealed that this small protein is secreted by an unknown mechanism into the cell walls of the micropylar region of the ovule and which is required of the final step of pollen tube guidance. Using ZmEA1 as bait we have identified a novel class of putative plant signalling molecules. Some members of this protein group, for example ZmEA1, contain predicted trans-membrane domains that might be cleaved by extracellular proteases. A candidate protease was identified in the egg cell library. *ZmES1-4* encode knottin-like proteins/peptides and seem to be secreted by the female gametophyte for pollen tube discharge. Finally, *ZmTLA1* encodes a novel plant peptide that might signal maternal tissues for degradation. We will report about gene discovery as well as functional and biochemical analyses of these female gametophyte expressed genes.

Cell-cell interactions during double fertilization

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Research in our laboratory focuses on the developmental genetics of plant reproduction using *Arabidopsis thaliana* and *Zea mays* as model systems. Using both genetic and molecular approaches we identified several genes controlling developmental processes underlying plant reproduction. In this presentation, I will focus on our work investigating cell-cell interactions during double fertilization. Unlike in animals, the gametes of plants do not differentiate directly from the meiotic products. Rather, the meiotic products divide mitotically to form the gametophytes, which in turn produce two pairs of gametes that participate in double fertilization. Over the last 10 years, we have undertaken several screens to identify genes involved in female sporogenesis, gametogenesis, double fertilization and maternal control of seed development. I will report on the molecular characterization of the *FERONIA* gene, defined by a female-specific mutant that led to the discovery of novel signaling processes between male and female gametophytes at fertilization. In *feronia* the pollen tubes, even if wild-type, are unable to release the sperm cells to effect fertilization (Huck et al., 2003). This phenotype suggests that the female gametophyte plays a crucial role in pollen tube reception and, thus, controls the behavior of the male gametophyte. The molecular nature of *FERONIA* is indeed consistent with a role in signal transduction. In addition to these cell-cell interactions at the level of male and female gametophytes we investigated interactions at the level of the gametes. Using maize as a model system, we could demonstrate that, surprisingly, the female gametes of plants do not have a block to prevent polyspermy.

REFERENCES

HUCK et al. 2003. *Development* 130: 2149–59.

Expressed gene products of the dimorphic sperm cells of *Plumbago zeylanica*

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An expressed sequence tag (EST) library was constructed for each of the two sperm cell types (S_{ua} and S_{vn}) of *Plumbago zeylanica* using populations of microinjector-collected sperm cells using the Clontech SMART library construction kit. The goal was to identify potential genes that may relate to their eventual cell fate, as it is known that the S_{ua} preferentially fertilizes the egg cell and creates the embryo, whereas the S_{vn} fertilizes the central cell and contributes to the formation of endosperm. Among S_{ua} library members, 893 sequences represented high quality nuclear gene products, 9 ribosomal products, 18 plastid products and 2 mitochondrial products (GenBank accessions: CB816827-CB817719). In the S_{vn} library, 629 sequences represented high quality nuclear gene products, 6 ribosomal products, 3 plastid products and 9 mitochondrial products (GenBank accessions: CB817720-CB818348). Of these recovered products, about half of the products were common to both libraries (46.9%), and slightly more unique products were found in the S_{ua} library (29.7%) than in the S_{vn} library (23 .4%). Differences between the two sperm cells were confirmed by microarray. Raw sequences are archived in GenBank and assembled into contigs representing unique products at URLs: http://www.genome.ou.edu/plumbago.html and http://bomi.ou.edu/russell/.

Unclassified proteins constituted 16.5% of the S_{ua} products and 14.9% of the S_{vn} products. There were no hits for 43.4% of S_{ua} and 46.9% of S_{vn} products. Presumably, angiosperm male gametes have a highly distinctive set of gene products that are not well represented in other tissues. Of classified genes, the largest category was "cellular processes", with "posttranslational modification and protein turnover" accounting for 20.7% S_{ua} and 29.4% S_{vn} products and "cell growth/division/chromosome partitioning" for 16.8% S_{ua} and 15.0% S_{vn} products. In the S_{ua}, "transcription" and "signal transduction" products were most differentially represented. In the S_{vn}, "posttranslational modification, protein turnover & chaperone activity" and "DNA replication, recombination & repair" were most differentially represented. *Plumbago* sperm cell products displayed a number of different patterns of expression. Frequently, gene products were expressed in sperm and in other tissues. Some gene products display apparent specificity for male tissues or sperm cells. Among sperm-expressed genes, many displayed nearly equal abundance in each sperm cell type, but there were also a number of gene products that showed a strong preference for a single sperm cell type, suggesting the likelihood of independent control and promoters for each sperm cell in *Plumbago*. A seeming anomaly is that gene products were difficult to find in generative cells of tricellular plants, suggesting early and late phase expression patterns may be quite distinct.

Regulatory genes that control embryo development

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In higher plants, seed development is initiated with a double fertilization event that gives rise to the diploid zygote and the triploid endosperm. The single-celled zygote then enters a phase of morphogenesis in which the basic body plan of the plant is established with fixation of the shoot-root axis, differentiation of the embryonic tissue systems, and formation of the shoot and root apical meristems and the embryonic organs. Late in embryogenesis, embryos enter the maturation phase in which they accumulate storage macromolecules and acquire the ability to withstand desiccation. To define genes that are required to make a seed and obtain insight into their roles in seed development, we have profiled RNA populations from *Arabidopsis* seeds at various stages of development. We have also characterized representative RNA populations at the other stages of the life cycle to identify genes expressed specifically during seed development and to determine the expression pattern of genes expressed at other stages of development. Our analysis of these data and use of the results to identify regulators of seed development will be discussed.

A number of different cell types in higher plants can undergo embryo development. In addition to zygotic embryogenesis, embryos can be formed through somatic embryogenesis, microspore embryogenesis, and a suite of processes collectively known as apomixis. To define mechanisms involved in the control of embryonic cell fate, we are studying the *Arabidopsis LEAFY COTYLEDON* (*LEC*) genes. The *LEC* genes, *LEC1*, *LEC2*, and *FUSCA3*, play essential roles in several aspects of embryogenesis. Moreover, ectopic expression of these genes induces embryonic characteristics and somatic embryo formation in vegetative cells. Insights into the mechanisms by which a cell is programmed to undergo embryogenesis will be discussed.

Legal framework for controlling GMOs

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Legal framework for controlling GMOs consists of two groups of legislations: international and national. The main piece of international law concerning GMOs is the Cartagena Protocol on Biosafety, which is a protocol to the Convention on Biological Diversity. The Cartagena Protocol provides a legal framework of a worldwide scale and a forum where both its 120 Parties (countries that have ratified the Protocol) and also non-parties (including the biggest biotechnology powers like USA and Canada) discuss issues of global importance for biosafety.

European Union considers biosafety one of its most important goal. There is an extensive set of Community legislations concerning GMOs with three basic Directives: Directive 90/219 on the contained use of GMOs, Directive 2001/18 on the deliberate release of GMOs, and Directive 98/44 on the legal protection of biotechnological inventions, as well as a number of Regulations. EU is also an important actor internationally promoting safe transfer, handling and use of GMOs based on precautionary principle.

Any legal framework concerning GMOs involves regulatory control of contained use, deliberate release into the environment and marketing GMO products, involving wide application of risk assessment, labeling and monitoring activities. Most legal schemes are complemented with public participation and access to information as well as liability and redress provisions.

Apical-basal axis formation in *Arabidopsis* early embryogenesis

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Embryogenesis transforms the fertilised egg cell into a multicellular organism. Plant embryos establish a main body axis of polarity that harbours stem-cell systems called shoot and root meristems at its opposite ends. In *Arabidopsis*, axis formation originates from the asymmetric division of the zygote. The apical daughter cell generates cluster of proembryo cells whereas the basal daughter cell makes a basal file of extraembryonic cells of which only the uppermost cell ("hypophysis") switches fate, initiating root meristem development. Molecular and cell-biological analyses suggest a model that links axis formation in early embryogenesis to polar auxin transport and response. The *GNOM* gene encodes a brefeldin A (BFA)-sensitive guanine-nucleotide exchange factor for an ARF GTPase (ARF-GEF) that mediates recycling of the putative auxin efflux carrier PIN1 to the basal plasma membrane, which is required for both embryo axis formation and lateral root initiation (Geldner et al., 2003; Friml et al., 2003; Geldner et al., 2004). A major focus of our current research is to identify mechanisms of ARF-GEF action in PIN1 recycling. The *BODENLOS* (*BDL*) gene encodes auxin-response regulator IAA12, which is thought to interfere with the activation of auxin-inducible target genes by the auxin-response factor ARF5 encoded by the *MONOPTEROS* (*MP*) gene. Although both genes are involved in the specification of the hypophysis, which initiates the formation of the primary root meristem, they are expressed in the adjacent proembryo cells rather than the hypophysis itself, suggesting that auxin-dependent cell-cell communication plays a role in root meristem initiation (Hamann et al., 2002). We are currently investigating mechanisms underlying the specificity of BDL-MP interaction.

REFERENCES

FRIML J, VIETEN A, SAUER M, WEIJERS D, SCHWARZ H, HAMANN T, OFFRINGA R, and JÜRGENS G. 2003. Efflux-dependent auxin gradients establish the apical-basal axis of *Arabidopsis*. *Nature* 426: 147–153.

GELDNER N, ANDERS N, WOLTERS H, KEICHER J, KORNBERGER W, MULLER P, DELBARRE A, UEDA T, NAKANO A, and JÜRGENS G. 2003. The *Arabidopsis* GNOM ARF-GEF mediates endosomal recycling, auxin transport, and auxin-dependent plant growth. *Cell* 112: 219–230.

GELDNER N, RICHTER S, VIETEN A, MARQUARDT S, TORRES-RUIZ RA, MAYER U, and JÜRGENS G. 2004. Partial loss-of-function alleles reveal a role for GNOM in auxin transport-related, post-embryonic development of *Arabidopsis*. *Development* 131: 389–400.

HAMANN T, BENKOVA E, BURLE I, KIENTZ M, and JÜRGENS G. 2002. The *Arabidopsis BODENLOS* gene encodes an auxin response protein inhibiting MONOPTEROS-mediated embryo patterning. *Genes Dev.* 16: 1610–1615.

Gene- and protein expression in isolated gametes, zygotes, apical and basal cells of the two-celled embryo of maize

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We focused on identifying genes that were up- or down-regulated in zygotes and in the apical or basal cell of two-celled maize embryos after in vitro fertilization in maize. A procedure for isolating apical and basal cells was established. Subsequently, cDNAs were synthesized from apical cells, basal cells, egg cells, two-celled embryos and multicellular embryos. These cDNAs were used as templates for randomly amplified polymorphic DNA (RAPD) PCR. Genes with specific expression patterns were identified, and these patterns were categorized into six groups: (1) Up-regulated only in the apical cell, (2) up-regulated only in the basal cell, (3) down-regulated only in the apical cell, (4) down-regulated only in the basal cell, (5) newly synthesized in both the apical and basal, (6) constitutively expressed in the egg cell and embryos. Genes up-regulated in the apical or basal cell (genes in groups 1 and 2) were already expressed in the early zygote, providing the possibility that transcripts from these genes are localized to the putative apical or basal region of the zygote, or that these are rapidly degraded in one of the daughter cells after zygotic division.

In another study, egg cell lysates were analyzed by polyacrylamide gel electrophoresis and subsequent mass spectrometry-based proteomics technology. Major protein components expressed in egg cell were identified: three cytosolic enzymes for the glycolytic pathway, glyceraldehyde–3-phosphate dehydrogenase, 3-phosphoglycerate kinase, and triosephosphate isomerase, two mitochondrial proteins, ATP synthase β-subunit and adenine nucleotide transporter, and annexin p35. Annexin p35 was highly expressed only in the egg cell, and glyceraldehyde–3-phosphate dehydrogenase, 3-phosphoglycerate kinase and the adenine nucleotide transporter were expressed at higher levels in egg cells than in central cells and cultured cells. These results suggest that annexin p35 in the egg cell and zygote is involved in exocytosis of cell wall materials, and that the egg cell is rich in an enzyme subset for the energy metabolism.

REFERENCES

OKAMOTO T, HIGUCHI K, SHINKAWA T, ISOBE T, LÖRZ H, KOSHIBA T, and KRANZ E. 2004. Identification of Major Proteins in Maize Egg Cells. *Plant Cell Physiology.* 45: 1406–1412.

OKAMOTO T, SCHOLTEN S, LÖRZ H, and KRANZ E. 2005. Identification of genes that are up- or down-regulated in the apical or basal cell of maize two-celled embryos and monitoring their expression during zygote development by a cell manipulation- and PCR-based approach. *Plant Cell Physiology* 46: 332–338.

Studies on autonomous embryo formation from Salmon wheat egg cells

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The 'Salmon' system of wheat provides a valuable experimental approach to study fertilisation-independent embryo formation. It comprises three isogenic alloplasmic lines with either zygotic (*aestivum* Salmon, aS) or autonomous, fertilisation-independent (*caudata* Salmon and kotschyi Salmon, cS and kS respectively) embryo development. While initiation of embryogenesis from isolated sexual egg cells depends on *in vitro* fertilisation, the corresponding parthenogenetic egg cell has the potential to undergo embryogenetic development without fertilization. Embryo formation from manually isolated cS and kS egg cells in vitro demonstrated that parthenogenesis is an inherent feature of this particular cell type. Based upon this observation, we have generated specific cDNA libraries from both sexual and parthenogenetic egg cells. 500,000 clones were obtained and 50,000 colonies robot-picked and arrayed per library. Test hybridisations with wheat chloroplast and mitochondrial DNA revealed a fairly high quality of the libraries. Further processing of the wild type egg cell library resulted in 2,800 sequenced clones and identification of about 30 putative egg cell-specific clones. Isolation and functional characterisation of the respective promoters will facilitate egg cell-specific over-expression and knock-down experiments using candidate parthenogenesis-related genes. Processing of the Salmon egg cell cDNA library is currently in progress.

Secreted molecules and their role in embryo formation in plants

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This short review emphasizes the importance of secreted molecules (peptides, proteins, arabinogalactan proteins, PR proteins, oligosaccharides) produced by cells and multicellular structures into the culture media. Several of these molecules have been also identified in planta within the micro-environment in which the embryo and the endosperm develop. Questions are raised about a parallel between the in vitro systems (somatic and androgenetic) and in planta zygotic development. A view of the exchanges between embryonic and non embryonic multicellular structures in vitro is described and several facts about the embryo and endosperm molecular interactions in planta are reported. It appears that the analysis of in vitro mechanisms may help the understanding of what happens during zygotic embryogenesis.

Somatic embryogenesis in *Arabidopsis*: Where do we go from here?

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The primary focus in much of the previous investigations on somatic embryogenesis in *Arabidopsis* was in increasing the yield of somatic embryos for biochemical and molecular studies. Somatic embryogenesis in *Arabidopsis* is routinely induced by culturing zygotic embryos in a medium containing 2,4-D to promote the formation of an embryogenic callus on the cotyledons and subsequent transfer of the callus to an auxin-free medium for the formation of somatic embryos. The origin of the embryogenic callus on the cotyledons has not been established with certainty although it has been proposed that cells present in the axils of cotyledons give rise to the callus. Study of somatic embryogenesis in a transgenic stock of *Arabidopsis* harboring a cyclin 1At::GUS construct to monitor the spatial and temporal patterns of cell cycling during culture of embryos in a medium containing 2,4-D has shown that cell divisions leading to callus formation are initiated in the procambial cells on the adaxial side at the base of the cotyledons. Later the entire cotyledon forms a compact callus on which early stage somatic embryos are produced. Transfer of the callus to an auxin-free medium results in the formation of mature-stage somatic embryos. When embryos subjected to increasing periods of 2,4-D action are grown in the auxin-free medium, they form in a transitional order normal seedlings, abnormal seedlings consisting of a short stem with ovate, spoon-shaped and bifid or lobed leaves and adventitious leafy shoots, callus of cotyledonary origin bearing a mixture of leaves and tubular somatic embryos with fused, bifid or lobed cotyledons, and finally callus with mature-stage tubular somatic embryos with fused bifid or lobed cotyledons. Based on these observations, somatic embryogenesis in *Arabidopsis* can be considered to occur in two distinctly separable stages. The first stage, which is labile, occurs after suboptimal periods of exposure of zygotic embryos to auxin when cells of the callus formed on cotyledons become potentially embryogenic and produce tubular somatic embryos with bifid or lobed cotyledons. These somatic embryos do not continue in the embryogenic pathway, but revert to a leaf-like morphology during subsequent periods of growth in an auxin-free medium. The second stage is the formation of mature-stage somatic embryos on the callus after a long period of exposure of zygotic embryos to auxin. Thus, the embryogenic identity of callus cells initiated by suboptimal periods of auxin action is stabilized only after an optimum period of exposure to 2,4-D, at the same time as the cells lose their leaf-like identity. The compact nature of the callus which does not allow for the production of a suspension culture containing single-celled progenitors of somatic embryos and the competing activities of two different genomes in the early-stage somatic embryos, one concerned with leaf formation and the other with the formation of somatic embryos are likely to limit the use of *Arabidopsis* as a model system to isolate genes that induce the embryogenic transformation of somatic cells.

Trafficking of pollen tube organelles along the cytoskeleton

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Organelle transport in eukaryotic cells is based on the dynamic interactions between cytoskeletal tracks and motor proteins, force-generating ATPases, which anchor to the surface of organelles through receptor proteins and drive them to specific cellular sites (Kamal and Goldstein, 2002). Evidence for the presence of myosins and microtubule-based motors on the same organelle raised the question of the interrelationships between the two motility systems (X). Analogously to other plant cells, the pollen tube shows an intense cytoplasmic streaming that is fundamental for the transport of molecules and organelles during its growth and that allows the pollen tube to achieve a uniform distribution of organelles and the relocation of the cytoplasm toward the growth region. The pollen tube exhibits a typical tip-growing mechanism that is dependent on the differential distribution of organelles and vesicles in the apical domain and is maintained essentially by the fusion of secretory vesicles with the apical plasma membrane (Derksen, 1996). A variety of evidences indicates that most of organelle movement occurs along actin filaments and is powered by organelle-associated myosins (Miller et al., 1995). Many uncertainties still exist on the role of microtubules in the active transport of pollen tube organelles and/or in the control of this process. Up to now, several microtubule-based motor proteins have been identified and characterized in the pollen tube of tobacco; nevertheless, information at the molecular level is scarce (Cai et al., 2001), as well as evidences on a possible functional cooperation between the two motility systems. In an effort to understand the molecular basis of microtubule-based movement, we extracted organelles from tobacco pollen tubes and analyzed their movement along in vitro-polymerized microtubules. We observed that different classes of organelles move along microtubules in the presence of ATP, in a way that is cytochalasin D- and cytosol-independent. This suggests that the movement does not require soluble factors and that microtubule-based motors are present on the organelle surface. After purification by selective binding to microtubules, organelle-extracted proteins were tested for the ability to glide microtubules under in vitro motility assay, for the presence of microtubule-stimulated ATPase activity and for cross-reactivity with anti-kinesin antibodies. These approaches allowed the characterization of a microtubule-based motor protein of 105 kD associated with the pollen tube organelles. The protein is therefore functionally, biochemically and immunologically related to kinesin (Romagnoli et al., 2003). These results indicate that tobacco pollen tube organelles move along microtubules and that microtubules could be involved in the transport of organelles in the pollen tube. The speed of motility induced by the microtubule system is far slower than that of streaming supported by the actin-myosin system in pollen tubes, suggesting that the microtubule-dependent transport of organelles may be masked by the rapid transport based on the actin-myosin system in pollen tubes. Until now, there is completely lacking evidence of a functional cooperation between microtubules and microfilaments in the transport of organelles and vesicles inside the pollen tube. In order to test the presence of functional cooperation between microtubules and actin filaments in the transport of pollen tubes organelles, we developed a method of double in vitro motility assay, which allowed us to observe the movement of pollen tube organelles along both microtubules and actin filaments on the same glass slide. We focused our attention on two classes of pollen tube organelles, mitochondria and secretory vesicles, which distribute and move differently inside the growing tube. Using immunoblotting analysis and binding assays to both microtubules and actin filaments, we identified kinesin-related proteins and myosins on the surface of pollen tube organelles. The double in vitro motility assays indicated that mitochondria and secretory vesicles show different speed and type of movement along microtubules and actin filaments. To sum up, these data suggest that plant mitochondria and secretory vesicles could have multiple types of motor proteins on their surface, which allow them to move along both actin filaments and microtubules.

REFERENCES

Kamal A, and Goldstein LSB. 2002. *Current Opinion in Cell Biology* 14: 63–68.

Derksen J. 1996. *Botanica Acta* 109: 341–345.

Miller DD, Scordilis SP, and Hepler PK. 1995. *Journal Cell Sciences* 108: 2549–2563.

Cai G, Romagnoli S, and Cresti M. 2001. *Sexual Plant Reproduction* 14: 27–34.

Romagnoli S, Cai G, and Cresti M. 2003. *Plant Cell* 15: 251–269.

Proteomics of anther and pollen development in tomato and *Arabidopsis*

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Anther and pollen development are essential developmental processes for sexual reproduction in angiosperms. We are interested in identifying proteins that are essential for these processes. The approach is to conduct a comparative proteomic analysis of Wild type (WT) and male-sterile mutants in two systems: 1) *7B–1*mutant in tomato that has a defect in pollen development at the pre-meoitic stage, and 2) *ms33* mutant in Arabidopsis in which pollen abortion occurs near maturity. The methodology involves isolation and separation of proteins by 1-D and 2-D gel electrophoresis, excision of protein spots, in-gel digestion, and analyses by matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) and/or liquid chromatography-electrospray ionization-quadrupole/time of flight tandem mass spectrometry (LC-ESI-Q/TOF-MS/MS). Peptide MS and MS/MS data were searched against publicly available protein and expressed sequence tag (EST) databases. As a first step, proteins in WT tissues were analyzed. The major proteins in WT tomato anthers at the pre-meoitic stage included, calreticulin-like and other calcium binding proteins, putative Pto-like serine/threonine kinase and capsid protein (defense related), translation initiation factor 5A–2, glycine-rich RNA binding protein, and proteins involved in electron transport. In the Arabidopsis WT mature pollen, the major proteins were also calcium binding and in addition, LEA proteins and other desiccation related proteins, stress signaling proteins (pp2c and Luminal binding), proteins involved with energy metabolism (ATP synthase, cytochrome c), cytoskeletal proteins (actin, profilin and tubulin binding) and translational factors were identified. The functions of various proteins in anther and pollen development will be discussed.

Role of genomic imprinting in endosperm development

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Genomic imprinting – the monoallelic expression of a small subset of genes depending on their parent of origin – is found in flowering plants and mammals. Various lines of evidence suggest that whilst imprinting in both phyla uses a common epigenetic mark provided by DNA methylation, the two employ radically different strategies in arranging for silence versus expression of imprinted genes.

Imprinting in mammals is a process of selective silencing and the default state is activity – silent alleles of imprinted loci are targeted for methylation of cytosine residues during gametogenesis, while expressed alleles generally remain relatively undermethylated. Silenced alleles are then protected from global demethylation during early embryogenesis to achieve monoallelic expression later in development. To support this process, mammals deploy a family of DNA methyltransferase proteins, including the maintenance methylase DNA METHYLTRANSFERASE 1 (DNMT1), as well as several de novo methylases.

In contrast to mammals, recent studies of imprinting in the model plant *Arabidopsis thaliana* suggest that the silent, hypermethylated state of imprinted genes is the default condition and that expression of maternal alleles requires a positive action – selective demethylation – on the part of the mother. The underlying mechanism appears relatively simple, requiring the antagonistic activities of two DNA modifying enzymes, DNA METHYLTRANSFERASE 1 (MET1) – the DNMT1 ortholog–, which maintains cytosine methylation, and DEMETER, a DNA glycosylase that may excise 5-methylcytosines.

Significantly, in this model imprint-specific DNA methylation does not require de novo methylases but the action MET1 alone, placing MET1 at the heart of the imprinting machinery. How this, and the other differences between imprinting in mammals and plants have arisen and their relationship to the life histories and evolutionary legacy of the two groups is the subject of this presentation.

Legal and social aspects of biotechnology development

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A value added chain that determines the economic growth consists of three basic factors:

VAC = [science and technology] + [legislation and patents] + [public awareness and participation]

In my belief these are the three basic elements that in parallel determine the future of biotechnology, especially modern biotechnology which is identified in practice with genetic engineering. The first is the scientific and technological progress, as innovative solutions create new possibilities. Next follows legislation, which is a form of social order regulation. At the end comes public opinion [the least valued in the past], virtually ignored by decision-makers and the society. The concept of harmonic biotechnology development is determined by many factors of fundamental significance:

- entrepreneurship and readiness to take justified social risk.
- the collaboration of scientific society with industrialists and financial circles,
- technological and laboratory base.
- financial possibilities and the availability of high risk capital.
- legal regulations, especially the protection of intellectual property.
- the support of state administration.
- public awareness and acceptance of innovative technologies.

The current situation in Poland, as in many other EU countries, invokes many objections and does not favour the dynamic development of biotechnology. Poland has a good chance to be not only and exclusively the consumer of biotech products but the producer as well.

Regulating cell division events during male gametophyte development

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The development of the haploid gametophyte generation that produces the male and female gametes occurs through relatively simple pathways involving only a few cell divisions. Although gametophyte development has been researched for well over a century little is known about the genes and the mechanisms that control cell division and how this is integrated with cellular differentiation. Our approach to this problem has been through pollen morphogenesis screens in *Arabidopsis*. These have led to the identification of a number of male gametophytic mutants with novel cellular phenotypes that affect asymmetric division (*gemini pollen),* cytokinesis *(two-in-one*) and cell cycle progression (*duo pollen*). A summary of progress that has been made in the analysis of these mutants and their corresponding gene products will be presented.

gem1 and *gem2* mutant alleles produce twin-celled pollen arising from disturbed microspore polarity (Park et al., 1998, 2004). GEM1 belongs to the Dis1/XMAP215 family of microtubule-associated proteins that stimulate plus-end microtubule growth. GEM1 binds to both cortical and mitotic microtubule arrays including the spindle and phragmoplast microtubules that strongly support its role in microtubule re-organisation during polarity establishment and cytokinesis at pollen mitosis I (Twell et al., 2002). In current work we are developing fluorescent markers to monitor gametophytic microtubule dynamics during polarity establishment, cytokinesis and cell cycle progression.

In contrast to *gemini pollen* mutants, *two-in-one (tio)* mutants do not affect microspore polarity and produce binucleate pollen grains arising from failure of cytokinesis at pollen mitosis I. Moreover, *tio* alleles also result in failure of cytokinesis in the embryo sac. Male gametophytic and somatic cell divisions involve 'conventional' modes of cytokinesis, whereas endosperm, meiotic and female gametophytic divisions rely on 'non-conventional' cellularisation after two or more cycles of mitosis (Otegui and Staehelin, 2000). We have identified TIO as a unique serine/threonine protein kinase in *Arabidopsis* and are currently investigating its localization, protein interactions and signalling role in conventional and non-conventional modes of cytokinesis.

We have isolated an entirely novel class of male-specific gametophytic mutants in *Arabidopsis* termed *duo pollen (duo)* that block division of the generative cell and provide compelling evidence for male germ line-specific control of cell cycle progression. Analysis of the nuclear DNA content and mitotic progression has revealed that two mutants (*duo1* and *duo3)* prevent transition between S and M phases of the generative cell cycle (Durbarry et al., 2005). Similar analyses of *duo2* demonstrates an essential role in mitotic progression at prometaphase of pollen mitosis II. *DUO1* encodes a unique R2R3 MYB protein in *Arabidopsis* that is specifically expressed in male germ line cells (Rotman et al., 2005) and DUO3 an unknown protein with acidic domains that also suggest its involvement in transcriptional regulation (Durbarry & Twell, unpublished). The *DUO1* promoter is sufficient to direct reporter gene expression to generative and sperm cells and is therefore a male-germ line-specific regulator. DUO1 and DUO3 proteins could act alone or in concert as transcriptional regulatory proteins required for generative cell cycle progression. In current work we are investigating the expression patterns, protein interactions and mechanism of action of DUO1 and DUO3 in generative cell cycle control.

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REFERENCES

DURBARRY A, VIZIR I, and TWELL D. 2005. Male germ line development in *Arabidopsis*: *duo pollen* mutants reveal gametophytic regulators of generative cell cycle progression. *Plant Physiology* 137: 297–307.

OTEGUI M, and STAEHELIN LA. 2000. Cytokinesis in flowering plants: more than one way to divide a cell. *Current Opinion in Plant Biology* 3: 493–502.

PARK SK, HOWDEN R, and TWELL D. 1998. The *Arabidopsis thaliana* gametophytic mutation *gemini pollen 1* disrupts microspore polarity, division asymmetry and pollen cell fate. *Development* 125: 3789–3799.

PARK SK, RAHMAN D, OH SA, and TWELL D. 2004. *Gemini pollen 2,* a male and female gametophytic cytokinesis defective mutation. *Sexual Plant Reproduction* 17: 63–70.

ROTMAN N, DURBARRY A, WARDLE A, YANG W-C, CHABOUD A, FAURE J-E, BERGER F, and TWELL D. 2005. A novel class of MYB factors controls sperm cell formation in plants. *Curr. Biol.* 15: 244–248.

TWELL D, PARK SK, HAWKINS TJ, SCHUBERT D, SCHMIDT R, SMERTENKO A, and HUSSEY PJ. 2002. MOR1/GEM1 plays an essential role in the plant-specific cytokinetic phragmoplast. *Nat Cell Biol.* 4: 711–714.

Recent embryological investigations of basket willow (*Salix viminalis*) as a source of biomass

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Recently, interest in *Salix* species has been increasing, because of using them as a source of alternative energy (Ahman and Larsson, 1994; Szczukowski et al., 2001; Weih, 2001). The research concerning the productivity and economical potential of *Salix* has mainly concentrated on *S. viminalis* and breeding programms have been implemented particularly by using interspecific crosses. Aspens are also acquiring increasing importance due to their ecological tolerance, wood quality and potentiality for biomass and energy production. There are no embryological informations on sexual reproduction of *Salix viminalis* and with the exception of only one short paper (Zenkteler et al., 2005) there are no documented studies on crossing *Salix* with *Populus*. Well documented informations on those topics may possess both fundamental and applied values. The presented paper will be focused on: (1) In vivo and in vitro self pollination of *S. viminalis*; (2) In vitro pollination of pistils and catkins of several clones of *S. viminalis* with pollen grains of *Populus tremula*, *P. alba*, *P. nigra*, *P. tomentosa*, *P. lasiocarpa* and *P. simonii*; (3) In vitro culture of ovules with hybrid globular embryos and isolated hybrid cotyledonary embryos; (4) development of hybrid seedlings.

Under the laboratory and in vitro conditions pollen grains of *S. viminalis* after selfing germinated abundantly, directed toward the lower part of the style and inside the embryo sacs. In 20 days, embryos with large cotyledons were fully formed. Under the same laboratory conditions pollen grains of poplars germinated sporadically on stigmas of *S. viminalis* but pollen tubes did not direct toward the ovary and embryos were never formed. Reversed processes occurred when catkins or single pistils cultured in vitro were covered with poplars' pollen grains. In all combinations of crosses pollen grains germinated abundantly and some pollen tubes were entering into the ovules.

Embryos at different stages of development have been observed between the third and twentieth day following pollination in all combinations of crosses. Some embryos disintegrated early at the globular stage as some other ones attained the cotyledonary stage. Seedlings were developing only from crosses *S. viminalis* × *P. nigra*, *P. tremula* and *P. simonii.* More details will be presenting on the development of hybrid embryos and hybrid seedlings.

REFERENCES

AHMAN I, and LARSSON S. 1994. Genetic improvement of willow (*Salix*) as a source of bioenergy. *Norvegian Journal of Agricultural Sciences* 18: 47–66.

SZCZUKOWSKI S, TWORKOWSKI J, and PIECHOCKI J. 2001. New trends of the utilisation of willow biomass harvested from arable land to energy generation. *Postępy Nauk Rolniczych* 6: 11–19.

WEIH M. 2001. Evidence for increased sensitivity to nutrient and water stress in a fast-growing hybrid willow compared with a natural willow clone. *Tree Physiology* 21: 1141–1148.

ZENKTELER M, WOJCIECHOWICZ M, BAGNIEWSKA-ZADWORNA A, ZENKTELER E, and JEŻOWSKI S. 2005. Intergeneric crassability studies on obtaining hybrids between *Salix viminalis* and four *Populus* species. *Trees* (in press).