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11th International Congress on Molecular Plant-Microbe Interactions held at St. Petersburg — A report

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The report is a short summary of the most interesting presentations at the 11th International Congress on Molecular Plant-Microbe Interactions held during July 18-27, 2003 at St. Petersburg, Russia. The key elements from several sessions on the legume-*Rhizobium* interactions have been discussed.

Keywords: Autoregulation, Calcium spiking, Legume, Nodule, Plant-microbe interactions, Proteomic analysis, *Rhizobium*

Legumes are very special plants. They form symbioses as a result of their roots being invaded by nitrogen-fixing soil bacteria, called rhizobia, which induce a root outgrowth called a nodule. The exchange of signals between the partners leads to specific developmental pathways being expressed. The plant provides carbon while the rhizobia produce fixed nitrogen which the legumes use for their metabolic needs. This report will only summarise key elements from several sessions on the legume-*Rhizobium* interactions.

How do legumes recognise and distinguish Rhizobium

The laboratory of Jens Stougaard[1] has used several new mutants of *Lotus japonicus* to generate new insights of the *Rhizobium*-legume interaction. Mutants *Ljsym1* and *Ljsym5* are really *nfr1* and *nfr5* and are LysM receptor kinases, known to interact with oligosaccharide repeat units. The *nfr5* gene is also *Pssym10* in pea and *nfr5* may be *Pssym2* of peas. Combining the data from both model legumes, *L. japonicus* and *Medicago truncatula*, a proposed signal transduction pathway might be: *nfr1*, *nfr5*, *symRK*, *dmi1*, *dmi2*, (Ca Spiking), *dmi3*, *nps1*, *nps2*, (ccd gene expression), *nin*, ... nodulation. The two nfr1 and nfr5 are receptor kinases and act before the NORK/ symRK gene activity. The cloned gene NORK is a receptor kinase (a *dmi2* ortholgue, G B Kiss) and is a member of a gene family called NSL (NORK Sequence Like) broadly distributed in the plant kingdom. One can imagine a cluster of receptor kinases at the root hair surface forming some type of heteromeric complex which in turn can bind the *Rhizobium* Nod Factors. The consequence of this binding could lead to ion fluxes, membrane depolarisation and ultimately NIN gene activation and the initiation of nodulation. However, it is the isolation of spontaneous nodulation mutants, that is nodulation in the absence of *Rhizobium* inoculation, that provides new insights to nodulation control [2]. It is mutant, *nar1*, which is a single recessive mutant that generates spontaneous nodules on *L. japonicus* at about the same levels as the wild type parent plant inoculated with *Rhizobium*. This has profound implications for control of nodulation. The *nar1* mutant will form Fix^+ nodules if inoculated with *Rhizobium* strains and it does show the autoregulation phenotype. It is in a new gene and the proposal is that *nar1* is a repressor of cortical cell division (ccd). This repression normally would be alleviated by *Rhizobium* inoculation and hence cortical cell division and nodule formation. The site of action of the repression system could be at the nucleic acid level where it would be inactivated by the *Rhizobium*-generated transduction signal or at the proposed kinase complex level. At this level the repression system might act as a phosphatase enzyme blocking the transmission of the phosphorylation of the signal transduction system. A second *nar* mutant, *nar2*, is a gain of function mutant which is dominant and produces a constitutive nodulation phenotype. Both these mutants can be regulated by added nitrate and illustrate the complexity of the multiple controls of ccd nodule primordia in legumes.

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The role of calcium

Another interesting discussion was about the role of calcium and the phenomenon of calcium spiking in the signalling transduction process during nodulation[3]. After the addition of Nod Factor to the root hairs an early event occurs within minutes which is the periodic oscillations of calcium levels within the hair cytoplasm, called calcium spiking[4]. This phenomenon is seen in a variety of indeterminate and determinate legumes and so it is considered as a component of the signal transduction system. Mutants blocked in the receptor or early steps in signal transduction are blocked in calcium spiking. Future experiments will be involved in attempting to biochemically link the initiation of the calcium spiking and the proposed kinase complexes of the cell surface and a possible linkage to the PLC, PIP, DAG, IP_3 pathways as seen in mammalian systems

Rhizobium colonisation and entry into root hairs

Once the rhizobia have bound to the root hair the problem then is entry into the hairs. Following the entrapment of the *Rhizobium* cells a trans-cellular tunnel is initated. Using a series of specific antibodies Nick Brewin[5] described his "inward force" model which results from a combination of the multiplication of the entrapped rhizobia and changes in the solid/fluid matrix that contains a glucoprotein from the family of glucoproteins which are gum-like in structure. These factors build up a pressure at the initiation site of a future infection thread. This development of a micro-colony of bacterial cells is probably part of the formation of biofilms and colonisation described below.

One of the most interesting new observations concerns the microsymbiont, rhizobia, and its ability to form biofilms and root colonisation. Many bacteria respond to changes in population density and coordinate the behaviour of individual cells in a local population through the exchange of extra-cellular signal molecules. This kind of regulation is called "quorum sensing" and affects many kinds of bacterial behaviour[6]. Quorum sensing appears to be particularly important in coordinating gene expression within a local bacterial population during its interaction with a eukaryotic host, during the differentiation of free-living bacteria into multicellular aggregates (biofilms)[7]. *Rhizobium* attachment to root hairs needs cell-to-cell contact. During studies of *Rhizobium* inoculation of transgenic lectin plants, the rhizobia were observed to form fimbriae, lots of EPS and biofilms (Hirsch[8]). Now such structures could provide protection for the bacteria, tolerance of antibiotics and facilitate gene transfer between cells within the biofilm. The biofilms on the roots formed towers and mushroom shapes[8]. By using different bacterial mutants it was shown that bacterial EPS was needed as the biofilms arrested in the micro colony stage, that *fil* mutants generated reduced tower structures and the addition of luteolin increased the formation of such structures. Moreover, the triple mutant *nodD1, D2, D3* did not produce the towers. The general proposal is that biofilm production is involved in root hair colonisation and so that would mean a role for quorum sensing signals in the infection process of nodulation.

Nodule development

In the early stages of nodule development in *Medicago* roots, the G0-arrested cortical cells de-differentiate and re-enter the cell cycle in front of the proto-xylem poles (Kondorosi *et al.*[9]). After several cycles, cell division is arrested and many cells undergo differentiation, endoreduplication and enlargement, leading to the establishment of nitrogen-fixing symbiosis. Many different sorts of genes are expressed in early nodule development such as the recently described ccs52A which is a novel regulator of cell cycle switch controls endoreduplication and late nodule development[9]. Antisense ccs52A transgenic plants have poorly developed nodules and changes in polyploidy. In addition, the nodule-specific gene families of GRPs (glycine-rich proteins) and the NCR group coding for at least 350 small polypeptides with conserved cysteine motifs. Studies of these groups show different expression times and expression profiles. Some of these NCR peptides can be as high as 4% of transcriptome. Their actual role in the developing nodule is still to be established.

A unique EMS-induced mutant, mutant *SGEapm*, described in peas[10] was impaired in shoot, leaf, flower and nodule development and makes a hybrid nodule structure which produces a lateral root growing out of the nodule. Initially, the nodule meristem develops normally but then generates a root meristem from part of the nodule meristem at the distal end of the nodule primordia. This observation implied a switch in direction of the committment of part of the meristematic cells and hence, a certain instability in the determination signals to the dividing plant cells of the meristem. Presumably this is happening throughout the plant in the various meristems and thus

the impaired shoot, leaf, flower structures. In crosses the gene segregates as a single recessive gene and so the *apm* gene is thought to specify meristem fate and is a gene controlling basic mechanisms of plant development.

Regulation of nodule number, the phenomena of autoregulation (ANO)

This area of research focuses on one of the major decisions made by any multicellular organism, i.e., when to allow cell division and when not to (Gresshoff[11]). The regulation of nodule numbers and mutants which alter this process were the subject of the soybean, *GmNARK*[11] and *Lotus japonicus Har1* (Hypernodulation aberrant root formation) (K. Szczyglowski[12]) systems. The *NARK/Har1/Sunn/Sym29* share a common sequence, essentially the same gene, are receptor kinases and are part of a receptor family that negatively control cell fate. Mutations in this gene control shoot-derived signals which have important implications for short and long distance regulation in legumes. The *GmNARK* gene is related to the *CLAVATA1* gene of *Arabidopsis*. Leaves from the soybean NARK mutant nts1007 were used in the rooted leaf assay (Rolfe and McIver[13]). It was found that the roots of younger leaves still form multiple nodules while the roots of older leaves nodulated like the roots on the wild type soybean. That is there is a developmental control in the autoregulation system. The *Har1* locus mutations alter root architecture by diminishing root length and increasing lateral root formation. Suppressor mutations of the Har1 phenotype were sought and several novel mutant types isolated. These were grouped into nodulating and non-nodulating isolates. Some had elongated roots, others short bushy roots, there were early abortion of infection mutants and others with hyper infection. Suppressors of the soybean NARK mutant have been found but are still uncharacterised. This area of plant developmental control of nodule cell growth promises to be very informative for all plant meristems.

Control of cell cycle during nodule development

The *Rhizobium* Nod factors activate the cell cycle in the G0-arrested cortical cells in front of the protoxylem poles in the nodulation sensitive root zone (E. Kondorosi[14]). This leads to the formation of a nodule primordium. An autonomous meristem is stablished in the apical region of the primordium while non-dividing submeristematic cells differentiate to various cell types in zone II of the nodule. Cells in this latter region do not divide but undergo several rounds of endoreduplication (G1-S-G2) cycles, the DNA content increases from 2C to 64C and the cells enlarge. Nod factors induce a CycA2:2 cyclin(A2-type) and auxin regulates it. This gene is required for the quiescent cortical cells re-activation and meristem formation. The function of CycA2:2 is strictly linked to cell proliferation, cell cycle switch gene ccs52A is needed for nodule differentiation. The gene ccs52A is essential for the exit from cell cycle activity and binds to an APC E3 ubiquitin ligases system. The gene ccs52A is necessary for nodule development and the formation of large highly polyploid cells is essential for nodule development.

Proteomic analysis of Sinorhizobium meliloti-Medicago truncatula interaction

Proteomic analysis which is the study of all accumulated proteins of an organism is a developing research tool in the post-genomic era. Such an analysis of *S. meliloti* strain 1021 using 2-D gel electrophoresis, peptide mass fingerprinting and bioinformatics showed that identification could be made of many of the gene products involved in: symbiosis, nutrient-stress specific reactions, normal free-living bacterial growth and the identification of several potential novel proteins not predicted from the DNA sequence (Djordjevic[15]).

Protein profiles of *M. truncatula* roots inoculated with various compounds and *Rhizobium* strains was examined by 2-D gel electrophoresis. More than 200 differentially accumulated proteins were identified by MALDI-TOF Mass Spectrometry using the *M. truncatula* EST and TC databases for peptide mass fingerprinting (Mathesius[16, 17]).

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