

lower in the IUGR group. Damage of proteins and DNA was slightly, but non-significantly higher in the IUGR group. Neonates with IUGR seem to have significant deficiency in antioxidant defense. IUGR is correlated with significant oxidative stress (Hracsko et al 2008).

Nitrogen monoxide (NO) is produced by nitric oxide synthases. The free radical nature of NO and peroxynitrite, renders NO a potent pro-oxidant molecule able to induce oxidative damage and potentially harmful toward cellular targets. Reactive nitrogen species modify amino acid residues, inhibit enzymatic activities, induce lipid peroxidation and deplete cellular antioxidant levels. These features may be associated with the development of different pathologies (Lyall et al. 1996) NO has diverse physiological roles and also known as a vasodilator.

We investigated the NO<sub>2</sub> and peroxynitrite level and the expression of eNOS by RT-PCR in the umbilical cord of IUGR neonates.

Our results support the hypothesis that increased NO production may be a compensatory response to improve blood flow in the umbilical cord. This increased eNOS expression and hence increased NO production in the fetal-placental vasculature may be an adaptive response to the increased resistance pathological pregnancies.

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## Characterization of a family of Arabidopsis receptor-like cytoplasmic kinases (RLCK class VI)

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Arabidopsis possess a large family of receptor-like kinases (RLKs) with more than 600 members (Shiu et al. 2004). Approximately 25% of the Arabidopsis RLKs contain only a kinase domain with no apparent signal sequence or transmembrane region and thus were collectively named as receptor like cytoplasmic kinases (RLCKs). Arabidopsis RLCKs can be subdivided into 10 classes with 193 protein coding genes altogether.

Concerning the function of plant RLCKs, at the present only few members have been characterized and it is very likely that they play major role in the perception and transmission of external signals perceived by RLKs (Zhou et al. 1995; Murase et al. 2004). Moreover, based on our previous investigations and recent literature data, we suppose that kinases belonging to RLCK class VI in Arabidopsis are Rop GTPase targets. Plant specific Rop GTPases are versatile molecular switches in many processes during plant growth, development and responses to the environment and thus a possible implication of RLCKs in these Rop-dependent signal transduction pathways is in discussion.

As part of our investigations related to Rop GTPase-mediated signal transduction in plants, we started to characterize the whole RLCK VI protein family in Arabidopsis. This is underway by studying the genes as well as the encoded proteins. A detailed analysis of the coding sequences and the gene expression pattern of all 14 RLCK\_VI members have already been accomplished. Sequence comparison and phylogenetic analysis revealed that gene duplication played a significant role in the formation of this kinase family and allowed the separation of the 14 RLCK VI kinases into two groups with seven members each (A1 to A7 and B1 to B7). It was established that, several members have an N-terminal UspA ("universal stress protein") domain (group B members) or an N-terminal serine-rich region (group A members) (Jurca et al. 2008).

In order to formulate a possible role of AtRLCK\_VI kinases, real-time quantitative reverse transcription-polymerase reaction (qRT-PCR) was used to determine relative transcript levels in the various organs (root, rosette leaves, cauline leaves, inflorescence stem, flower buds, open flowers, siliques, exponentially dividing cultured cells) of the Arabidopsis plant as well as under a series of abiotic stress/hormone (osmotic, sugar, salt stress, oxidative stress, cold and hormone treatment) treatments in seedlings. The obtained data revealed the differentially regulated expression of the genes, which is in agreement with a high variability of sequence elements in their promoter regions. Thus, the encoded kinase proteins may be involved in a wide variety of signal transduction pathways related to plant development and stress responses (Jurca et al. 2008).

After characterizing the expression of the At-RLCK VI genes, it was imperative to study the proteins itself to find a possible function of these cytoplasmic kinases. Our previous data as well as recent publications indicated that some of the RLCK\_VI members can interact with Rop GTPases. Therefore we decided to establish an RLCK\_VI-to-Rop interaction matrix including 10 members of both families (4 RLCK\_VI and one Rop genes could not be cloned due to various reasons) using the yeast two-hybrid system. As controls, RLCK class IV, VII and IX members as well as alfalfa RLCK\_VI kinases and Rop GTPases were also involved. In general it could be stated that members of RLCK\_VI group A showed interaction with several Rops while that of group B not. The biological role of this interaction needs to be determined. In this direction we further proceed with the in vitro characterization of the activity of these kinases as well as with the produc-



tion of transgenic plants over-expressing or silencing RLCK\_VIA genes. The identification of altered phenotypes in these transgenic plants can be very helpful in order to determine the developmental role of RLCK class VI members in Arabidopsis.

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## Structural analysis of antimicrobial peptides by molecular dynamics methods

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Cationic antimicrobial peptides (AMPs) play an important role in the innate immune system. There are several experimental methods for investigating the secondary structures of these small molecules but they are not precise enough to provide reliable information. Accordingly, we chose molecular dynamics methods to investigate the structural properties of some AMPs. Three types of peptides were studied: peptides rich in His (alloferon-1 and -2), peptides rich in Trp and Arg (indolicidin and tritrypticin) and cyclic peptides containing a disulfide bridge (bactenecin and tigerinin-1).

Alloferon-1 and -2 isolated from insects are rich in His and they possess antiviral and antitumor activities with immunomodulatory effect (Chernysh 2002). The secondary structure of alloferons has not been examined yet. Indolicidin and tritrypticin are peptides containing aromatic residues isolated from bovine neutrophils (Selsted 1992; Lawyer 1996). They possess broad spectrum of antibacterial, antifungal and hemolytic activities. Both indolicidin and tritrypticin are known to be flexible in aqueous solution and adopt either helical (poly-proline II helix) or turn structures in membrane mimic environment. Bactenecin and tigerinin-1 are cyclic peptides with serious antimicrobial activity (Romeo 1988; Sai 2001). Bactenecin was isolated from bovine neutrophils and tigerinin-1 was isolated from the skin of *Rana tigrina*. Each of them tends to adopt  $\beta$ -turn conformation. Because of the controversial assumptions and the lack of reasonable information about the secondary structures of these AMPs our goal was to perform conformational analysis of these peptides.

To explore the conformational spaces of molecules simulated annealing calculations were performed using implicit solvent model. For peptides containing Pro residues, torsional restraints were applied to keep the Xxx-Pro peptide bonds either in *cis* or *trans* configurations. The evolving secondary structures and the intramolecular interactions were examined.

For indolicidin and tritrypticin, it was observed that the *cis-trans* isomerisation plays a key role in the distribution of secondary structural elements (Kerényi 2007). For *trans* isomers, mainly type I and III  $\beta$ -turns were identified. Nevertheless,  $3_{10}$ - and poly-proline II helical segments also appeared along the sequence of peptides possessing *trans* Xxx-Pro peptide bonds. In *cis* isomers, type VI  $\beta$ -turns were observed in specific tetrapeptide units. The stabilizing intramolecular interactions were in good agreement with the structural data: the observed H-bonds play a role in the stabilization of type I and III  $\beta$ -turns, as well as of  $3_{10}$ -helical segments, while the proline-aromatic interactions participate in the stabilization of type VI  $\beta$ -turns. In alloferons, type I, II, II' and III  $\beta$ -turns were the most frequent structural elements. These secondary structures were also stabilized by backbone H-bonds. In the cyclic peptides (bactenecin and tigerinin-1), type I and III  $\beta$ -turns could be found in major population. In the *cis* isomers of tigerinin-1, type VI  $\beta$ -turns were also identified. In every peptide examined, minor populations of backbone-sidechain and sidechain-sidechain H-bonds were also found.

The results obtained from modelling the secondary structures and stabilizing intramolecular interactions were coherent and the conclusions derived from these calculations coincided with the data published so far.

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