

We have developed and apply a microarray-based method to identify germ plasm enriched RNA-s. We performed a series of experiments on different microarray platforms to compare the RNA content of numerous germ plasm-less, germ plasm overexpressing and wildtype conditions. Collating our datasets with the list of the known germ plasm enriched transcripts, we found that germ plasm overexpressing vs. wildtype comparison is the most appropriate method to identify new germ plasm enriched transcripts. In such comparisons 380 transcripts showed at least four times increase in germ plasm overexpressing condition. These transcripts were chosen for further analysis to confirm their germ plasm localization by making use of fluorescent RNA in situ hybridization (Lecuyer et al. 2007) on early *Drosophila* embryos. To be able to accomplish such a large number of in situ hybridisations we have developed a suitable PCR based single strand DNA labeling method.

Another approach we used, is a network based identification of novel germ plasm factors. We built up and investigated a germ plasm specific gene interaction network. First, we searched RNA localization databases (BDGP, Fly-FISH) and original publication for genes whose transcripts are exclusively or highly enriched in the germ plasm (Szuperák et al. 2005). This way, 136 as we called "original" germ line specific genes were found. Then we identified their primary genetic and yeast two-hybrid interactors by using the BioGRID database. Based on the GEO database, those primary interactors which are not expressed at early embryonic stages were filtered out. Finally, we constructed a gene interaction network which indicates all known interactions (325) among the original germ line specific factors (136) and primary interactors (325). We assume that the number of interactions of a given gene may mirror its importance in the network. Genes with large number of interactions, also called hubs, can refer to a central role of a given gene that have a good chance to show phenotype when it is mutated. We confirm this hypothesis by RNAi induced phenocopy analysis. We are currently analyzing the germ line specific phenocopies of a representative group of hubs as well as of the low connectivity control genes. The phenocopy of the RNA silencing is followed by the time laps video microscopy which allows distinguishing different type phenocopies: the complete absence or decreased number of germ cells, or its migration defects.

BDGP, Patterns of gene expression in *Drosophila* embryogenesis. <http://www.fruitfly.org/cgi-bin/ex/insitu.pl>

BioGRID, General repository for Interaction Datasets. <http://www.thebiogrid.org/>

Fly-FISH, A Database of *Drosophila* embryo mRNA localization patterns. <http://fly-fish.cabr.utoronto.ca/>

GEO: Gene Expression Omnibus. <http://www.ncbi.nlm.nih.gov/geo/>: GEO Accession: GSE3955

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Oxidative stress, intrauterine retardation, modes of delivery

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Oxidative stress arises when the balance between oxidants and antioxidants is disturbed. The source of free radicals is the unpaired electron of molecular oxygen, which makes it unstable and electrically charged. In the lack of antioxidant molecules and enzymes, free radicals target lipids, proteins and DNA. Oxidative damage to DNA is a result of interaction of the nucleic acid with hydroxyl radical that generates strand breaks on the DNA. Oxidative stress is a physiological event in the fetal-to-neonatal transition.

The steadily increasing global rate of cesarean sections (CS) has become one of the most debated topics in maternity care. The mode of delivery may have a considerable effect on the state and health of the newborn. CS is a surgical intervention with potential hazards for both mother and child. The opinions of obstetrician-gynecologists regarding normal vaginal delivery (VD) and CS are highly contradictory. The results of previous studies display great differences. We have approached this question from a consideration of oxidative stress and set out to determine a wide range of parameters relating to the oxidative status of neonates born via VD or undergoing CS.

We conclude that the mode of delivery does not have a serious effect on the level of free radical damage if there is no emergency situation. The elective CS does not have an advantage over VD with respect to oxidative stress (Hracsko et al. 2007).

Intrauterine growth retardation (IUGR) is a complication of pregnancy. A newborn with IUGR weighs less than do 90% of all other newborns of the same gestational age. The reported incidence of IUGR ranges between 7 and 10 per cent. This abnormality is associated with increased level of morbidity and mortality, and deformation of the umbilical cord.

The mechanism of development of IUGR has still not been appropriately described, although it is most probably a consequence of an abnormal fetomaternal blood circulation. Accordingly we have carried out examinations on umbilical cord blood and endothelium in order to establish how the antioxidant status of full-term IUGR infants changes and whether the results indicate significant oxidative stress. We compared the antioxidant status and the level of lipid peroxidation (LP) of the umbilical blood in healthy mature neonates and in IUGR neonates. The level of LP was high in the IUGR group while the antioxidant enzyme activities and the levels of antioxidants were significantly

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 NO₂ 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-