ABCG driven transport of phenylpropanoids in *Medicago truncatula* - a versatile but adapted scenario.

Medicago truncatula is not only rapidly developing model crop plant for studying legumes biology but also an excellent species for the studies on secondary metabolism of legumes. Particular interest is in phenylpropanoids. The utility of phenylpropanoids is a matter of being in the right place at the right time. This is tightly controlled, not only at the biosynthesis level, but also by various distribution systems. The latter comprise, inter alia, membrane transporters which participate in the circulation of both the intermediates and final products. Revealing the transporters/transport mechanisms/determinants involved in/behind dedicated translocation will not only bridge the knowledge gaps regarding spatiotemporal phenylpropanoid production under various conditions, but will also facilitate more precise metabolic engineering of those compounds in plants, in order to improve agronomic traits or nutritional value.

Among transporters involved in phenylpropanoid distribution are ABC proteins. The latter form the largest known protein family with more than 1000 members operating in all living cells from bacteria to man. In most cases, functional ABC transporters act as membrane ATP-driven pumps. It is postulated that plant ABC transporter genes underwent multiplication and functional diversification/specialization, and thereby assumed the ability to transport selected compounds critical for successful adaptation to dry land, notably secondary metabolites. The ABCG subfamily is the largest among ABC proteins and ABCGs are especially numerous in plants. Plant ABCGs are linked to secondary metabolites distribution, hormone transport and cell detoxification. They have been also described as being involved in dedicated response to biotic stresses.

Our laboratory identified 30 full size ABCG membrane transporters in *Medicago truncatula*. Twice as much as e.g. in Arabidopsis. Conducted phylogenetical studies reveled that certain Medicago ABCGs form expanding and distinctive for legume clades. With this project we aim to provide, in particular, biochemical and structural information about ABCG advancement and evolutional/functional adaptation of these clades members to individual Medicago needs. We would like to take into account already acquired knowledge about Medicago ABCG transporters as well as a special/dedicated role of certain phenylpropanoids in legumes. Our hypothesis is that almost identical Medicago ABCGs are not simply redundant but they are functional, specialized and translocate different/dedicated molecules. By broad and multiple analyses (*in silico* modelling, molecular biology, reverse genetics, transport experiments) we want also to determine a sequential feature connecting transporter with a given molecule(s) and at the same time understand/propose the meaning of such an adaptation regarding distribution of endogenous molecules. Results of this study might represent an added value to the effort aiming deciphering determinants liable for ABC(G) transporters action in legumes. Such data could also facilitate more precise metabolic engineering of phenylpropanoids in plants often relying on defined spatiotemporal distribution.

Based on our previous experience in functional characterization of ABCGs from other plant species we propose that plant ABCGs offer matchless chance to look for determinants (single amino acids or even α helices) responsible for the molecular fit between protein and a particular class of secondary metabolites/translocated molecules. It is wort to emphasize that the molecular mechanisms/determinants that influence transport fulfilled by full size ABCGs are not fully understood. We do believe that careful implementation of bioinformatics analyses, molecular dynamics, together with functional characterization (expression, localization) and especially transport experiments dedicated to this phylogenetically selected group of transporters will help also to answer the key/intriguing question: are we able to predict *in silico* possible protein/transported molecule relationship? This is important since even though ABCG transporters have been shown to play various and crucial roles in plants, only a few of them have been characterized from the functional and substrate points of view. Often the exact substrates transported by most ABC transporters are still debated or unknown. The reason is that only a limited number of studies have used proposed targeted *in silico* strategy to identify substrates of plant ABC transporters.