Summary

In this thesis, the regulation of transport is investigated as a limiting factor in biosynthesis of plant secondary metabolites. CjMDR1 in *Catharanthus roseus* (L.) G. Don. has been selected as a model system of active transporters. CjMDR1 is a member of ABC (ATP-Binding Cassette) protein superfamily. Especially, in previous reports it was found to transport preferably berberine in its producing plant, *Coptis japonica*. In order to look into the role of the ABC transporter berberine was exogenously fed to *Catharanthus roseus* cell line and evaluate the transport of the compound. Based on a series of experiments in this study it can be concluded as follows.

1. Transport of berberine in wild *Catharanthus roseus* was not clearly associated with ATP-dependency. Using diverse inhibitors it was proved that berberine was not an exclusive substrate of native ABC transporters in *Catharanthus*. Alternatively berberine might be transported via H^+-antiport or efflux transporters from MATE (multidrug and toxic compound extrusion) group which was previously found in other heterologous plants (Yazaki, 2005). In reality in *Catharanthus* some effect of pH on plasmatic membrane on berberine transport has been observed. However, the exact mechanism of berberine transport on vacuolar and cell level was not fully uncovered yet.

2. Transgenic cell lines of *Catharanthus roseus* have been prepared by particle bombarding method (biolistic method). Inserted gene was originally isolated from *Coptis japonica* and encodes plasmatic ABC transporter.

3. Both chemical inducible and constitutively expressing transgenic lines of *Catharanthus roseus* were tested for the presence of the transgene. Signal of the transgene was detected only by PCR, not by Northern or Western blotting technique. Genomic DNA as well as cDNA of transgenic lines was assayed and the presence of the transgene proved. Fusion of CjMDR1 with GFP was preceded and the transporter seems to be considerably localized in plasmatic membrane of *Catharanthus roseus.*

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4. Among all the evaluated transgenic lines, MDR-7 displays significantly higher accumulation of ajmalicine and tetrahydroalstonine in comparison with transgenic control and wild type cells. Increased accumulations of serpentine and catharanthine were observed only against transgenic control line, not against wild type cells. On the other hand, berberine transport shows quantitatively the same rate in wild type, transgenic and transgenic control lines.

Substrate specificity of an ABC transporter in plant culture via transgenic approach can not be easily evaluated. The gene of ABC transporter is rather big and all applied molecular biology techniques need several optimization steps, particle bombarding techniques does not show as high sufficiency as in Tobacco case (Ondřej a Drobník, 2002) and besides protein tests, entire

transport experiments might be corrupted by native *Catharanthus* ABC transporters. Yeast (*Saccharomyces cerevisiae*) cells with corrupted Yeast cadmium factor 1 (Ycf1p) and possibly also Bpt1p seems to be the good alternative for expression of ABC transporters. Yeast has six MRP ABC members, however after corrupting the above mentioned genes, no ATP dependent GS-X transport activity is detected, which makes this stream ideal system for substrate study of ABC transporters (Sharma *et al.*, 2002; Klein *et al.*, 2002).

However, according to Kutchan (2005b), the biggest challenge of metabolic engineering nowadays is: what kind of tool shall we use to analyze our experiments? Microscope or telescope? Maybe the best would be to use both zooms at the same time. Thus study made on model systems with minimum external factors involvement might not be exactly transposable

to real eukaryotic culture with all sophisticated regulation and strict organization.