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Improved accumulation of ajmalicine and tetrahydroalstonine in *Catharanthus* cells expressing an ABC transporter

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Summary

The biosynthetic pathway of monoterpenoid indole alkaloids in *Catharanthus roseus* is located throughout various membranes at both the cellular and intercellular levels. ATP-binding cassette (ABC) transporters are known to export vincristine and vinblastine from human cancer cells. It has recently been shown that ABC transporters are also involved in the transport of various monoterpenoid alkaloids in *Catharanthus roseus* cells. Over-expression of an ABC transporter in this plant might therefore affect the regulation of the alkaloid biosynthetic pathway. CjMDR1, an ABC transporter gene originally isolated from *Coptis japonica*, was expressed in *Catharanthus roseus* cell cultures. Cells showing a positive PCR signal of the transgene in both cDNA and genomic DNA samples were subject to transport studies using selected substrates. Unexpectedly, transport of the isoquinoline alkaloid berberine, the main substrate of CjMDR1 transporter in *Coptis japonica*, was not

Abbreviation: ABC, ATP-binding cassette; DW, dry weight; MDR, multidrug resistance proteins; MIA, monoterpenoid indole alkaloids; MRP, multidrug resistance associated proteins; WT, wild-type cells.

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affected as compared with control and wild-type *Catharanthus* cells. On the other hand, the endogenous alkaloids ajmalicine and tetrahydroalstonine were accumulated significantly more in *Catharanthus roseus* cells expressing CjMDR1 in comparison with control lines after feeding these alkaloids.

Introduction

In the past years, *Catharanthus roseus* has become a model plant for secondary metabolism studies. This is due to the presence of monoterpenoid indole alkaloids (MIA) with antitumor as well as other valuable biological activities (van der Heijden et al., 2004). Decades of *Catharanthus* research in fields varying from molecular biology to enzymology and chemistry have resulted in an impressive body of information (just within the 2007–2008 period, approximately 19 reviews and many more experimental papers have been published, e.g. El-Sayed and Verpoorte, 2007; Facchini and De Luca, 2008; Hisiger and Jolicoeur, 2007; Jaleel et al., 2008; Lee-Parsons, 2007; Loyola-Vargas et al., 2007). However, our present understanding of the formation of MIA is still not sufficient to overcome the main stumbling block in commercial production—a low yield of MIA in both field-grown plants and *in vitro* cultured *Catharanthus* cells.

One of the potential factors in biosynthetic pathway regulation is transport. As known from previous studies, biosynthetic pathways leading to the bis-indole alkaloids include at least five compartments at the subcellular level (plastids, vacuoles, cytosol, microsomes, endoplasmatic reticulum) and three different cell types (reviewed in Facchini and De Luca, 2008; Facchini, 2001; Roytrakul and Verpoorte, 2007; Yazaki et al., 2008). The key enzymes involved in the biosynthesis are not widespread throughout the entire plant, but occur in high concentrations only in selected tissues (St-Pierre et al., 1999). Consequently, intermediates are translocated in order to reach their target compartment. Thus, the influx and efflux from the cells and the cellular compartments could constitute very important regulation sites (Johansen et al., 2006; Kunze et al., 2002).

The transport in cells can be divided into two groups based on energy dependence: passive and active transport. Passive transport and diffusion are dependent on concentration gradients and physical parameters. Passive transport results in equal partitioning of all compounds through all cells and cellular compartments. On the other hand, the active transporters are responsible for the accumulation of certain compounds in certain cells or cellular compartments. Three major active transport systems occur in membranes; ATP-binding cassette (ABC) transporters and two proton pumps: H^+-ATPase (V-ATPase, P-ATPase, F_1/F_0-ATPase) and H^+-pyrophosphatase (V-PPase, H^+-PPase) (Gaxiola et al., 2007; Rea and Sanders, 1987). Previous studies using bean hypocotyls reported that, within all proteins isolated from the tonoplast, about 40% comprise aquaporines, 15% V-ATPase and 10% V-PPase (Higuchi et al., 1998; Maeshima and Yoshida, 1989; Matsuura-Endo et al., 1990). Although ABC transporters are not listed as the most abundant, they seem to play an important role. ABC proteins represent a large gene family and have been found to mediate transport of various substrates across membranes in prokaryotes and eukaryotes (Gottesman et al., 1996; Higgins, 1992). In humans, members of the ABC family of transporters are the main reason for the failure of anti-cancer therapy (Gottesman et al., 2002), since the transporters participate in exclusion of chemotherapeutics such as colchicine, paclitaxel, vincristine, vinblastine, doxorubicine and adriamycine from the cells (Dean et al., 2001). The function of ABC transporters in plants remains largely unknown and is at present extensively studied (Burke and Ardehali, 2007; Crouzet et al., 2006; Geisler and Murphy, 2006; Klein et al., 2006; Morris and Zhang, 2006; Rea, 2007; Roytrakul and Verpoorte, 2007; Schulz and Kolukisaoglu, 2006; Shitan and Yazaki, 2007; Wanders et al., 2007; Yazaki, 2006). Yazaki et al. (2001) isolated a gene encoding an ABC transporter (CjMDR1) from *Coptis japonica*. The isoquinoline alkaloid berberine and its biosynthetic precursor are exclusive substrates for the CjMDR1 transmembrane protein, which does not have (at least according to what is known at present) such a broad substrate specificity as many other known plant ABC transporters (Shitan et al., 2003). The aim of this study was to test substrate specificity of CjMDR1 in connection with MIA produced in *Catharanthus roseus*. CjMDR1 was expressed in *Catharanthus roseus* cells and the changes in accumulation of selected MIA were measured. The results of this study should lead to possible novel strategies for stimulation of MIA biosynthesis by over-expression of ABC-genes.