Involvement of ABC proteins in the transport of endogenous low molecular weight metabolites in plants

Kazuyoshi Terasaka
Laboratory of Plant Gene Expression, RISH, Kyoto University

Alkaloids comprise one of the largest groups of secondary products, and have divergent chemical structures and diverse biological activities. Plant alkaloids are used in both modern and traditional medicine, e. g., vincristine and taxol are prescribed as anticancer drugs, morphine and scopolamine are used as analgestics, and berberine, a benzylisoquinoline alkaloid, is conventionally used as an antibacterial and antimaleria drug. These alkaloids also often show strong cytotoxicity to prokaryotic and eukaryotic cells; e. g., vincristine inhibits microtubule formation, and berberine inhibits DNA and protein synthesis. Because of these activities, alkaloids are presumed to play an important role as a biological barrier to protect the plant tissue from pathogens. Indeed, berberine shows strong antimicrobial activity to both Gram-positive and Gram-negative bacteria as well as other microorganisms.

On the other hand, alkaloid-producing plant cells seem to be insensitive to their metabolites. Berberine-producing plant cells must have a mechanism protecting them from the cytotoxicity of berberine, although how it is detoxified in plant cells is still unclear. One of such detoxification mechanisms is probably the sequestration of berberine into vacuoles or the efflux of berberine by the plant cells to keep apart it from the cytosol and also from the nucleus. We have been studying the alkaloid transport mechanism in berberine-producing cultured cells of *Thalictrum minus* and *Coptis japonica* as model systems to elucidate the detoxification mechanism.

I have demonstrated in this study the high tolerance of cultured *T. minus* cells that produce a large amount of berberine, in comparison with cells that do not have berberine biosynthetic pathway. While cultured *T. minus* cells preferentially excreted the endogenous berberine into the medium, they also excluded berberine exogenously added to the culture, as well as a heterocyclic dye, neutral red, and calcein AM, a fluorescent probe to measure the pumping activity of multidrug efflux transporter in an ATP-dependent manner. Further analyses using inhibitors of ATP-binding cassette (ABC) proteins have suggested that a member of ABC transporters is involved in the transport of berberine in the *Thalictrum* cells [1].

In order to identify plant ABC proteins, I applied the vanadate-induced nucleotide trap, which is conventionally used as an effective analytical technique to characterize mammalian ABC-type drug efflux pumps. ABC proteins show drug-stimulated ATPase activity, and the catalytic sites have low affinity and specificity for nucleotides. The catalytic site is stably labeled with 8-azido-ATP by UV when the activity of ABC proteins is inhibited by vanadate. This study showed that this technique could be applicable to plant cells for characterizing ABC proteins expressed in berberine-producing cell cultures.

Thus, the vanadate-induced nucleotide trapping technique was applied to berberine-producing plant cell cultures, *T. minus* and *C. japonica*. One membrane protein at ca. 180 kDa was photoaffinity-labeled with 8-azido-[α-^{32}P]ATP in the *T. minus* cells in the presence of vanadate, which was specifically induced by the addition of benzyladenine in a similar manner as the induction of berberine biosynthesis in these cell cultures, whereas three bands were observed in the *C. japonica* cells in the size region between 120 and 150 kDa corresponding to full-sized ABC protein [2]. The benzyladenine-induced band in *T. minus* showed properties similar to those of human MDR1, including the recognition of berberine, which suggests that the ABC protein detected in *T. minus* takes this endogenous alkaloid as a putative substrate for transport. This was the first application of this technique to plant cells.

To address more general questions, what kind of role plant ABC proteins are playing, I chose a model plant *Arabisopsis thaliana*, and found 22 members that belong to MDR subfamily among *ca.* 130 ABC protein genes in the genome. The most resemble members with *Thalictrum* MDR type ABC transporter have been analyzed on gene expression level, as well as the functional level in Arabidopsis

REFERENCES

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