
**Activity of the purified plant ABC transporter NtPDR1 is stimulated by diterpenes and sesquiterpenes involved in constitutive and induced defenses.**


**Summary**

Within the plant ABC transporter family, pleiotropic drug resistance (PDR) transporters play essential functions, such as in hormone transport or defense against biotic and abiotic stresses. NtPDR1 from Nicotiana tabacum has been shown to be involved in the constitutive defense against pathogens through the secretion of toxic cyclic diterpenes such as the antimicrobial substrates cembrene and sclareol from the leaf hairs (trichomes). However, direct evidence of an interaction between NtPDR1 and terpenes is lacking. Here, we stably expressed NtPDR1 in N. tabacum BY-2 suspension cells. NtPDR1 was purified as an active monomer glycosylated at a single site in the third external loop. NtPDR1 reconstitution in proteoliposomes stimulated its basal ATPase activity from 21 to 38 nmol Pi.mg-1.min-1, and ATPase activity was further stimulated by the NtPDR1 substrates cembrene and sclareol, providing direct evidence of an interaction between NtPDR1 and its two substrates. Interestingly, NtPDR1 was also stimulated by capsidiol, a sesquiterpene produced by N. tabacum upon pathogen attack. We also monitored the transcriptional activity from the NtPDR1 promoter in situ with a reporter gene and found that while NtPDR1 expression was limited to trichomes under normal conditions, addition of methyl jasmonate, a biotic stress hormone, induced expression in all leaf tissues. This finding indicated that NtPDR1 is involved not only in constitutive but also in induced plant defenses. In conclusion, we provide direct evidence of an interaction between the NtPDR1 transporter and its substrates and that NtPDR1 transports compounds involved in both constitutive (diterpenes) and induced (sesquiterpenes) plant defenses.


**Cell-free reconstitution reveals centriole cartwheel assembly mechanisms.**

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**Summary**

How cellular organelles assemble is a fundamental question in biology. The centriole organelle organizes around a nine-fold symmetrical cartwheel structure typically ∼100 nm high comprising a stack of rings that each accommodates nine homodimers of SAS-6 proteins. Whether nine-fold symmetrical ring-like assemblies of SAS-6 proteins harbour more peripheral cartwheel elements is unclear. Furthermore, the mechanisms governing ring stacking are not known. Here we develop a cell-free reconstitution system for core cartwheel assembly. Using cryo-electron tomography, we uncover that the Chlamydomonas reinhardtii
proteins CrSAS-6 and Bld10p together drive assembly of the core cartwheel. Moreover, we discover that CrSAS-6 possesses autonomous properties that ensure self-organized ring stacking. Mathematical fitting of reconstituted cartwheel height distribution suggests a mechanism whereby preferential addition of pairs of SAS-6 rings governs cartwheel growth. In conclusion, we have developed a cell-free reconstitution system that reveals fundamental assembly principles at the root of centriole biogenesis.


Summary

Pleiotropic drug resistance (PDR) transporters belong to the ABCG subfamily of ATP-binding cassette (ABC) transporters and are involved in the transport of various molecules across plasma membranes. During evolution, PDR genes appeared independently in fungi and in plants from a duplication of a half-size ABC gene. The enzymatic properties of purified PDR transporters from yeast have been characterized. This is not the case for any plant PDR transporter, or, incidentally, for any purified plant ABC transporter. Yet, plant PDR transporters play important roles in plant physiology such as hormone signaling or resistance to pathogens or herbivores. Here, we describe the expression, purification, enzymatic characterization and 2D analysis by electron microscopy of NpABCG5/NpPDR5 from Nicotiana plumbaginifolia, which has been shown to be involved in the plant defense against herbivores. We constitutively expressed NpABCG5/NpPDR5, provided with a His-tag in a homologous system: suspension cells from Nicotiana tabacum (Bright Yellow 2 line). NpABCG5/NpPDR5 was targeted to the plasma membrane and was solubilized by dodecyl maltoside and purified by Ni-affinity chromatography. The ATP-hydrolyzing specific activity (27 nmol min(-1) mg(-1)) was stimulated seven-fold in the presence of 0.1% asolectin. Electron microscopy analysis indicated that NpABCG5/NpPDR5 is monomeric and with dimensions shorter than those of known ABC transporters. Enzymatic data (optimal pH and sensitivity to inhibitors) confirmed that plant and fungal PDR transporters have different properties. These data also show that N. tabacum suspension cells are a convenient host for the purification and biochemical characterization of ABC transporters.

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Summary

Mapping the conformational landscape of G protein-coupled receptors (GPCRs), and in particular how this landscape is modulated by the membrane environment, is required to gain a clear picture of how signaling proceeds. To this end, we have developed an original strategy based on solution-state nuclear magnetic resonance combined with an efficient isotope labeling scheme. This strategy was applied to a typical GPCR, the leukotriene B4 receptor BLT2, reconstituted in a lipid bilayer. Because of this, we are able to provide direct evidence that BLT2 explores a complex landscape that includes four different conformational states for the unliganded receptor. The relative distribution of the different states is modulated by ligands and the sterol content of the membrane, in parallel with the changes in the ability of the receptor to activate its cognate G protein. This demonstrates a conformational coupling between the agonist and the membrane environment that is likely to be fundamental for GPCR signaling.