COMMENTARY

New wine in an old bottle: ABCB19, known as auxin exporter, also exports brassinosteroids

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Brassinosteroids (BRs) constitute a fundamental group of phytohormones that regulate plant growth and development, orchestrating responses to various environmental stimuli. BRs are synthesized within the cell interior but bind to receptors at the cell surface, suggesting the presence of a yet unidentified export mechanism. A recent study by Ying et al. (2024)[1] shows that a member of the ATP-binding cassette (ABC) transporter superfamily and the known auxin transporter, ABCB19, also functions as a BR transporter. This finding is supported by induction of ATPase activities, binding and transport assays, structure, and mutant phenotypes in *Arabidopsis*. The identification of this BR transporter provides a new clue for modulating BR homeostasis to adapt to dynamic environmental changes, and improves our understanding of the roles of ABCB subgroups.

Hormone movement and redistribution are critical for maintaining phytohormone homeostasis. Regulating hormone responses requires transportation across various levels, including the subcellular, cellular, and tissue levels. Recent studies have identified several transporter subfamilies responsible for transporting all known canonical hormones, with the exception of BRs. These include ABC family proteins, nitrate transporter/peptide transporter (NPF) proteins, multidrug and toxic compound extrusion (MATE)-type transporters, PIN-FORMED (PIN) proteins, AUXIN RESISTANT1 (AUX1) and LIKE AUX1 (LAX), purine permease (PUP), Sugars Will Eventually Be Exported Transporters (SWEET) family,[2] among others. However, researchers have long been puzzled by the discovery of BR transporters, as their biosynthesis sites are different from the sites where the signals are perceived. Drawing inspiration from the export of steroid hormones by ABCBs in mammalian cells and observing that the *abcb1,19* double mutant exhibits a phenotype more resembling BR deficiency phenotype[3] than the typical auxin-deficient phenotype[4] (Figure 1A), Ying et al. boldly raise the question: do ABCB19 and ABCB1 have additional substrates apart from auxin?

The authors conducted several critical assays to unequivocally demonstrate that ABCB19 functions as a BR exporter. Initially, they performed a quantitative analysis revealing a dose-dependent induction of ABCB19's ATPase activity upon BR treatment, achieving a near four-fold increase at 10 μM BR concentration compared to the mock control. Subsequently, they performed both in vitro and in vivo transport assays, directly demonstrating ABCB19's involvement in BR export. Using cryo-electron microscopy (cryo-EM), they monitored the structural changes in ABCB19 when bound to BRs, thereby visually confirming its BR efflux function. Genetic analysis, combined with ABCB1, revealed that the *abcb1,19* mutant exhibited phenotypic traits similar to BR-deficient plants. This further strengthened the role of ABCB19 in BR signaling. Furthermore, they discovered that the abundance of ABCB1 and ABCB19 on the plasma membrane was regulated by BRs. Experiments with exogenous application of BR or the BR biosynthesis inhibitor brassinazole (BRZ) further corroborated the positive regulation of BR signaling by ABCB19, which in turn influenced the turnover of the transporter.

This groundbreaking study unveils a novel role of ABCB19, providing compelling evidence that the expression levels of *ABCB19* or *ABCB1* significantly influence BR signaling. The discovery marks a significant breakthrough in identifying BR transporters, a long-overlooked component of BR transport models. The findings strongly suggest that ABCB19 plays a pivotal role in maintaining BR homeostasis, thereby regulating BR signaling and transduction, ultimately influencing plant growth and development. This novel insight into the complex mechanisms of BR signaling and transport represents a significant advancement in the field of plant biology and could potentially lead to new strategies for optimizing plant growth and productivity.

The re-definition of ABCB19 as a BR exporter after being widely recognized as an auxin exporter for so long raises intriguing questions. This shift can be understood from various perspectives. First, advancements in technology, particularly in cryo-EM, has enabled researchers to obtain the crystal structure of membrane-localized proteins and capture their different conformations under varing states, facilitating a deeper understanding of their functional roles. Second, previous studies on hormone transport often focus on the cellular level, whereas this study delves into the molecular level. By analyzing the biochemical, structural, and genetic aspects of ABCB19, researchers are able to demonstrate its direct involvement in BRs transport. Third, the spirit of inquiry in research prompts researchers to challenge conventional thinking and explore alternative hypotheses. The identification of ABCB19 as a BR exporter is a prime example of this inquisitive approach, highlighting the importance of questioning established paradigms.

Such revelation prompts further exploration into the roles of other ABCB transporters, such as ABCB15-22,[2,5] ABCB6 and ABCB20,[2] which have also been identified as auxin exporters but exhibit phenotypes resembling BR-deficient phenotype (Figures 1A-B). This opens avenues for the research community to delve deeper into the roles of ABCBs and uncover potential novel transport mechanisms for new substrates (Figure 1C). Continual investigation in this area holds the promise of uncovering new insights into hormone transport and signaling pathways.

There are several fascinating aspects within this study that merit deeper investigation. One key question is to understand why a single transporter, like ABCB19, can accommodate multiple substrates. Another crucial one is whether the phosphorylation of ABCB19 R domain regulates its structural dynamics and substrate specificity. Understanding the mechanisms that govern how ABCB19 switches between transporting auxin and BRs is also paramount. In addition, investigating the link between the two hormones and elucidating how ABCB19 selects between them could provide valuable insights into hormone signaling pathways. Intriguingly, while ABCB19 has been shown to export both auxin and BRs, it is unclear why only BR treatment activates its ATPase activity. This discrepancy calls for a deeper investigation into the molecular basis of ABCB19's response to different hormones. Furthermore, while ABCB19 has been identified as a BR exporter at the cellular level, there is still a need for further investigation into other BR transporters at the subcellular and tissue levels that are involved in long-distance transport. However, it is also a matter of debate whether long-distance BR transport actually occurs.

In conclusion, the identification of ABCB19 as a BR exporter provides profound insights into how BRs are exported at the cellular level to positively regulate BR signaling. This discovery offers a novel perspective on the multifaceted role of ABCBs as hormone transporters, suggesting that these transporters may have diverse substrates. Finally, the revelation of ABCB19's novel function will motivate further in-depth investigations into the functions and underlying mechanisms of other ABCB subfamily transporters.

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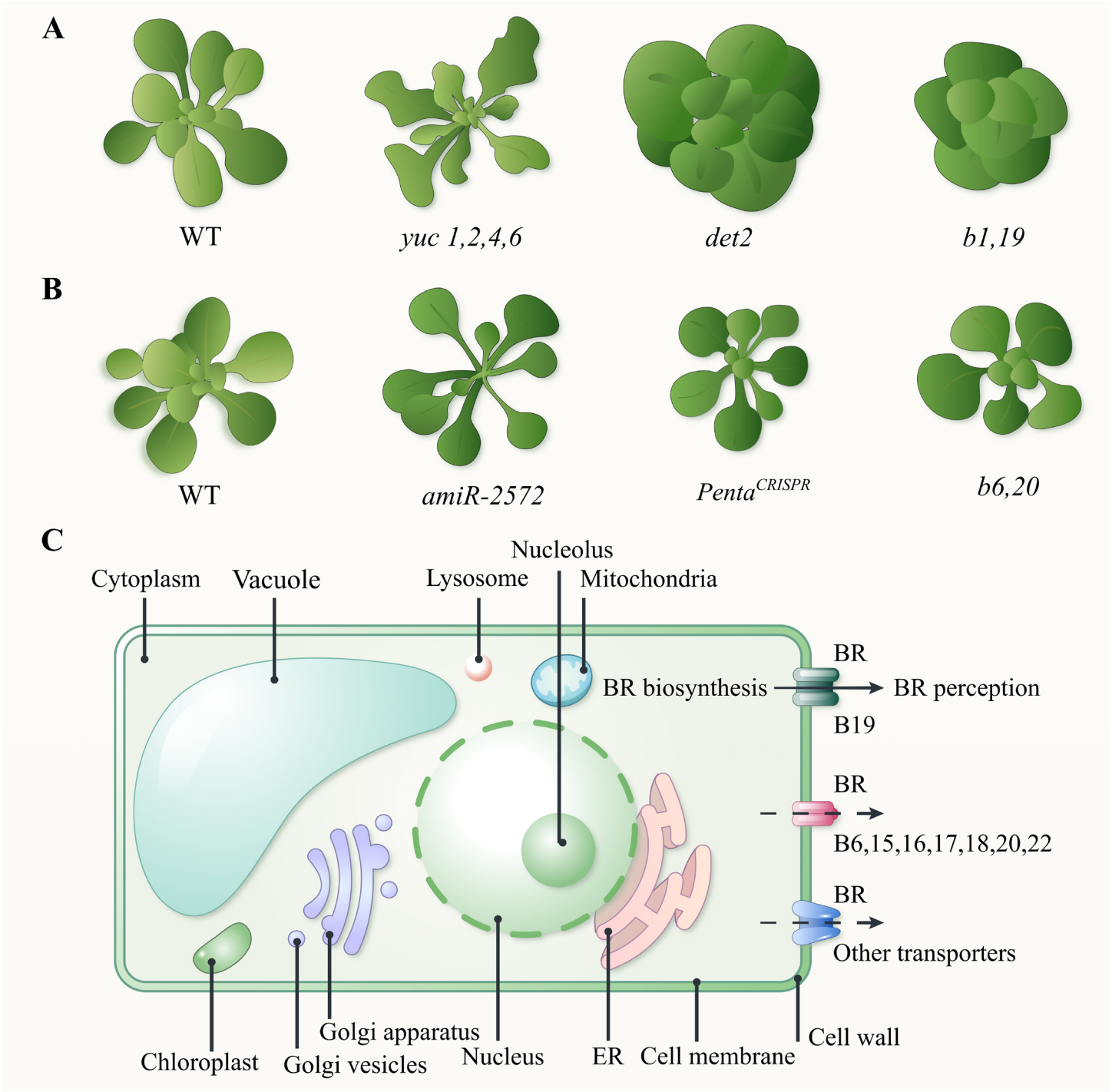

Figure 1. Illustrations depicting potential brassinosteroids transporters in *Arabidopsis* (A) Illustrations of shoot phenotypes for WT, *yuc1,2,4,6*, *det2*, and *abcb1,19* (*b1,19*) mutants. (B) Illustrations of shoot phenotypes for WT, *amiR-2572* (targeting *ABCB16,17,18,22*), *Penta*^{CRISPR} (targeting *ABCB15,16,17,18,22*), and *abcb6,20* (*b6,20*) mutants. (C) A model of ABCB19 exporting brassinosteroids (Solid arrow line) and the potential BRs transporters (Dashed arrow line).

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AUTHOR CONTRIBUTIONS

Y.D. and Y.Z. were responsible for conceptualization and writing the original draft. Y.D., N.S., and Y.Z. wrote and edited the manuscript. Y.Z. made the figure and supervised this work. All authors contributed to the article and approved the submitted version.

DECLARATION OF INTERESTS

The authors declare no competing interests.