Plant Science 245 (2016) 1–10

Contents lists available at ScienceDirect

Plant Science

journal homepage: www.elsevier.com/locate/plantsci

Review article

Master and servant: Regulation of auxin transporters by FKBPs and cyclophilins

Markus Geisler[a,*], Aurélien Bailly[b], Maria Ivanchenko[c,*]

[a] University of Fribourg, Department of Biology—Plant Biology, CH-1700 Fribourg, Switzerland
[b] University of Zurich, Institute of Plant Biology, CH-8008 Zurich, Switzerland
[c] Oregon State University, Department of Botany and Plant Pathology, 2082 Cordley Hall, Corvallis, OR 97331, USA

ARTICLE INFO

Article history:
Received 1 October 2015
Received in revised form
14 December 2015
Accepted 17 December 2015
Available online 16 January 2016

Keywords:
Immunophilins
Cyclophilins
FK506-binding protein (FKBP)
Auxin transport
Protein–protein interaction
Chaperon

ABSTRACT

Plant development and architecture are greatly influenced by the polar distribution of the essential hormone auxin. The directional influx and efflux of auxin from plant cells depends primarily on AUX1/LAX, PIN, and ABCB/PGP/MDR families of auxin transport proteins. The functional analysis of these proteins has progressed rapidly within the last decade thanks to the establishment of heterologous auxin transport systems. Heterologous co-expression allowed also for the testing of protein–protein interactions involved in the regulation of transporters and identified relationships with members of the FK506-Binding Protein (FKBP) and cyclophilin protein families, which are best known in non-plant systems as cellular receptors for the immunosuppressant drugs, FK506 and cyclosporin A, respectively. Current evidence that such interactions affect membrane trafficking, and potentially the activity of auxin transporters is reviewed. We also propose that FKBPs andcyclophilins might integrate the action of auxin transport inhibitors, such as NPA, on members of the ABCB and PIN family, respectively. Finally, we outline open questions that might be useful for further elucidation of the role of immunophilins as regulators (servants) of auxin transporters (masters).

Contents

1. Introduction

Numerous developmental processes in multicellular organisms rely on the establishment of tissue polarities [1]. Spatial developmental information in plants is conveyed in part through the directional distribution of the essential hormone, auxin [2]. Auxin accumulation and its directional distribution among neighboring cells, referred to as polar auxin transport, represent the core of the ability of auxin to elicit differential effects on plant growth and development [1]. In this manner, polar auxin transport is a primary mechanism in the regulation of plant cell physiology and development [1]. Therefore, auxin transport has been a matter of extensive interest and investigation ever since the emergence of the auxin concept more than a century ago [3]. Although seemingly a simple problem, it turned out to be very difficult to rigorously address the physiology and function of auxin transport proteins at the molecular level [4]: auxin transport studies have been found to be complicated by the diffusion component of auxins ([5,6]; see Section 2 and Box 1). Moreover, membrane proteins are generally difficult to analyze functionally because of their low solubility at conditions that preserve their native structure and function. Thus,

* Corresponding authors.
E-mail addresses: markus.geisler@unifr.ch (M. Geisler), ivanchem@science.oregonstate.edu (M. Ivanchenko).

http://dx.doi.org/10.1016/j.plantsci.2015.12.004
0168-9452/

Box 1: Diffusion versus primary and secondary active auxin transport in plants.
Much of the complex structure of biological membranes is dedicated to the regulation of solute transport. Passive diffusion of ions and other hydrophilic molecules across the hydrophobic cellular membranes is low. The transport of substrates that cannot diffuse freely across membranes, moreover their directional movement against a concentration gradient, as in the case of polar auxin transport, requires energy. Primary transporters are fueled in most cases by ATP hydrolysis whereas secondary transporters are driven by tapping the electrochemical gradients existing across biological membranes [24]. These are usually Na^+ gradients in animal cells provided by the action of Na^+/K^+-ATPases or H^+ gradients in fungi and plants generated by the action of H^+-ATPases on the plasma membrane, respectively. Channels, in contrast, when open let ions diffuse rapidly down electrical and concentration gradients resulting in detectable currents. ABCBs were clearly shown to utilize ATP as a direct energy source and to transport auxin steadily across cellular membranes, suggesting ABCBs act in most cases as primary auxin pumps [4,6,127]. PINs and AUX1/LAXs are thought to act as secondary active auxin exporters dependent on an electrochemical gradient. AUX1/LAXs are similar to bacterial permease-like amino-acid transporters [23] that most likely act as H^+/IAA symporters [24]. The energization of PINs is less clear and hypothesized based on the fact that PINs do not possess ATP-binding domains [128]. Interestingly, complex functional interactions among some PINs and ABCBs have been reported [19–21], however, the biological relevance and mechanism of these interactions are far from being understood.

the energy-coupling mechanisms and activity regulation of auxin transporters have remained poorly understood. In contrast, significant progress has been made during the last several years in understanding the membrane targeting of auxin transporters and their effects on plant development [5]. Some advances have been also made toward the elucidation of the functional interactions of auxin transporters with additional regulatory proteins of the immunophilin class. These may affect auxin-transporter trafficking and/or activity [7–10]. The progress in this more recent field is summarized and critically discussed here.

2. Auxin transport across biological membranes

According to the chemiosmotic model of auxin transport [11–14], a substantial portion of IAA is protonated in the apoplast and able to enter cells *via* bilayer diffusion, whereas IAA inside the cells is less protonated and its efflux requires active transport. As to our knowledge, whether this is completely so has never been proven experimentally. In this context it is worth mentioning that drug leakage into cells by lipophilic diffusion versus hitchhiking of transporters is currently highly debated in the animal literature [15].

To date, three major families of membrane localized proteins involved in auxin transport have been characterized in *Arabidopsis* and other plant species: PINs, named after the *pin-formed1* (*pin1*) Arabidopsis mutant, ABCBs (ATP-binding cassette, type B) and AUX1/LAXs (AUXIN1/LIKE AUXIN1) [5,16]. Mutations in *PIN1* and combined mutations in other *PIN* genes with PIN1 result in organogenesis defects, indicating that PINs mediate directional auxin flow that regulates organogenesis [17,18]. In contrast, *abcb*1, 19 mutants although significantly dwarfed display only subtle morphological defects [19,20] suggesting that ABCBs function primarily in export of auxin out of meristematic tissues with high auxin concentrations, and in maintenance of long-distance auxin flows required for physiological processes (reviewed in Refs. [4,21]).

AUX/LAX have been discussed to generate auxin sinks as a driving force for auxin transport streams in the stele in the context of lateral root development [22]. The contribution of AUX1 in generating auxin sinks is in agreement with the total loss of gravitropic responses in *aux1* [23].

Depending on the direction of transport, the systems are termed influx transporters and efflux transporters. Members of plasma membrane located AUX1/LAX family are grouped as auxin importers [24]. Auxin exporters are primarily represented by plasma membrane localized PINs (so-called long PINs, see below) and members of the ABCB family, which have so far all been found to be plasma membrane-embedded [6,25–27]. However, it should be mentioned that recently ABCB4 and ABCB21 were characterized as facultative importer/exporters whose transport directionality seems to be triggered by intracellular auxin levels [28].

The regulation of plasma membrane presence and polarity of auxin transporters has been studied extensively. Decent progress has been also made in respect to individual transporter regulation by protein degradation, phosphorylation and protein–protein interaction (for reviews, see Refs. [7,29,30]). Long PINs (PIN1–4,7) comprising a large cytoplasmic loop [5] are often polarly localized to a specific face of the plasma membrane in Arabidopsis root cells, in good correlation with the direction of the auxin flow [31]. By contrast, short PINs (PIN5,6,8) are localized in the endomembrane system (most likely the endoplasmic reticulum (ER)) and appear to function in intracellular auxin transport and homeostasis [32,33]. Recently, a second family of ER-localized auxin transporters, called PILS (for PIN-LIKES), was also shown to regulate auxin homeostasis [34], although their role in auxin transport is less clear. AUX1/LAX and ABCB proteins are plasma membrane localized like long PINs, but are most often apolar (reviewed in Ref. [1]).

All classes of plasma membrane-based auxin transporters are brought to their specific membrane domains by vesicle trafficking along actin tracks [35]. However, based on different sensitivities to brefeldin A the cycling of ABCBs and PINs as well as that of individual members of each family, respectively, seems to reveal specific trafficking mobilities as summarized in excellent reviews [35,36].

Recent analyses in heterologous, non-plant auxin-transport systems (such as yeast, HeLa cells and Xenopus oocytes) demonstrated that AUX1/LAX influx, and ABCB and PIN-mediated IAA efflux activity is substrate-specific and rate limiting [6,19,25,26,37–42], providing experimental evidence that these proteins are *bona fide* auxin transporters. Although plant ABCBs belong to the large superfamily of multidrug resistance transporters, they were found to own a high degree of substrate specificity toward only a few auxinic compounds but not to transport closely related substances (such as the anti-auxin 2-NAA or benzoic acid [6]) or classical substrates of mammalian ABCBs (such as rhodamin123, daunomycin and vinblastine [43]). ABCB-mediated auxin transport was dependent on ATP hydrolysis [6,37] as expected for primary active pumps, and sensitive to inhibitors of auxin efflux (such as NPA, and flavonols [6,37,43,44]) along with known inhibitors of mammalian multidrug ABC transporters (such as cyclosporine A and verapamil [6,43]). Recently, expression of ABCB19 in HeLa cells was also reported to result in anion currents that were inhibited by applying an anion channel blocker [45]. Currently it is unclear if this indicates a unique ion channel function for ABCB19, ion channel function additional to pump function, or an artifact of the heterologous expression. Furthermore, it is unknown whether these currents represent IAA^- fluxes. It is worth mentioning though that ion channel function has been described for some mammalian ABC transporters [46]. It has also been reported that co-expression of ABCB and PIN combinations leads to synergistic transport rates and alters substrate specificities and inhibitor sensitivities [20,21]. The individual roles