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ABP1 Mediates Auxin Inhibition of Clathrin-Dependent Endocytosis in *Arabidopsis*

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SUMMARY

Spatial distribution of the plant hormone auxin regulates multiple aspects of plant development. These self-regulating auxin gradients are established by the action of PIN auxin transporters, whose activity is regulated by their constitutive cycling between the plasma membrane and endosomes. Here, we show that auxin signaling by the auxin receptor AUXIN-BINDING PROTEIN 1 (ABP1) inhibits the clathrin-mediated internalization of PIN proteins. ABP1 acts as a positive factor in clathrin recruitment to the plasma membrane, thereby promoting endocytosis. Auxin binding to ABP1 interferes with this action and leads to the inhibition of clathrin-mediated endocytosis. Our study demonstrates that ABP1 mediates a nontranscriptional auxin signaling that regulates the evolutionarily conserved process of clathrin-mediated endocytosis and suggests that this signaling may be essential for the developmentally important feedback of auxin on its own transport.

INTRODUCTION

The plant signaling molecule auxin is an important regulator of plant developmental processes, including embryogenesis, organogenesis, tissue patterning, and growth responses to external stimuli (Santner and Estelle, 2009; Vanneste and Friml, 2009). Current models on auxin signaling and action focus on the paradigm that auxin regulates the expression of subsets of genes, thus eliciting different cellular and, consequently, developmental responses. Nuclear auxin signaling involves the F box protein transport inhibitor response 1 (TIR1), which acts as an auxin coreceptor (Kepinski and Leyser, 2005; Dharmasiri et al., 2005a, 2005b; Tan et al., 2007), and downstream Aux/IAA and ARF transcriptional regulators (Dharmasiri and Estelle, 2004). This pathway controls a remarkable number of auxin-mediated processes, but some rapid cellular responses to auxin are not associated with TIR1-based signaling (Badescu and Napier, 2006; Schenck et al., 2010).

Decades ago, the plant-specific protein AUXIN-BINDING PROTEIN 1 (ABP1) was proposed to be an auxin receptor (Hertel et al., 1972; Löbler and Klämbt, 1985). ABP1 in both monocot and dicot plant species shows physiological affinities toward natural and synthetic auxin ligands (Jones, 1994). ABP1, despite carrying a KDEL-endoplasmic reticulum (ER) retention motif, is secreted to some extent to the extracellular space where it is active (Jones and Herman, 1993; Tian et al., 1995; Henderson et al., 1997). ABP1 is essential for embryogenesis (Chen et al., 2001) and postembryonic shoot and root development (Braun et al., 2008; Tromas et al., 2009) and mediates auxin effect on cell elongation, but the underlying mechanism remains unclear (Jones et al., 1998; Leblanc et al., 1997).

An important regulatory level in auxin action is its differential distribution within tissues (Vanneste and Friml, 2009). Such auxin gradients result from local auxin biosynthesis and directional,

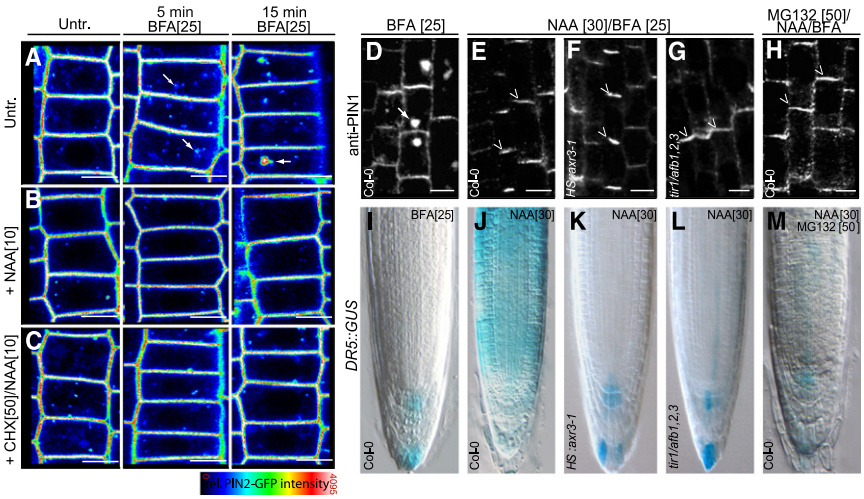

Figure 1. Auxin-Mediated Inhibition of Endocytosis by Nontranscriptional, TIR1-Independent Mechanism

(A–C) Time lapse showing BFA-induced increase of PIN2-GFP endosomal signal and its intracellular accumulation within minutes (A). NAA treatment effectively and rapidly inhibits BFA-induced PIN2-GFP internalization (B) also when protein synthesis is inhibited by cycloheximide (CHX) (C).

(D–H) BFA treatment for 90 min induces intracellular accumulation of PIN1 (D). Auxins, such as NAA (30 min pretreatment), inhibit BFA-induced PIN1 internalization in the wild-type (E); in the TIR1-mediated auxin signaling-deficient mutants, such as overexpressors of stabilized IAA17 (*HS::axr3-1*; induced for 2 hr at 37°C) (F); in the *tir/afb* quadruple mutant (G); and after MG132-mediated inhibition of proteasome function (H). See also Figure S1.

(I–M) Auxin treatments for 3 hr, such as NAA (J), but not BFA alone (I), induce transcriptional auxin response monitored by *DR5::GUS* in the wild-type (J), but not in the *HS::axr3-1* (K) and *tir/afb* quadruple (L) mutants or after MG132 treatment (M). See also Figure S1.

Arrows mark PIN proteins internalized into BFA compartments. Arrowheads highlight PIN retention at the plasma membrane. Scale bar, 10 μm.

intercellular auxin transport (Petrásek and Friml, 2009) that is triggered by a network of carrier proteins (Swarup et al., 2008; Geisler et al., 2005; Petrásek et al., 2006; Vieten et al., 2007; Yang and Murphy, 2009). The directionality of auxin flow depends on the polar plasma membrane distribution of PIN-FORMED (PIN) auxin efflux carriers (Wiśniewska et al., 2006). In addition to PIN phosphorylation that directs PIN polar targeting (Friml et al., 2004; Michniewicz et al., 2007), PIN activity can be regulated by constitutive endocytic recycling from and to the plasma membrane (Geldner et al., 2001; Friml et al., 2002; Dhonukshe et al., 2007). Auxin itself inhibits the internalization of PIN proteins, increasing their levels and activity at the plasma membrane (Paciorek et al., 2005). The molecular mechanism of this auxin effect remains unknown, but it has been proposed to account for a feedback regulation of cellular auxin homeostasis and for multiple auxin-mediated polarization processes (Leyser, 2006). Here, we show that auxin regulation of PIN internalization involves the ABP1-mediated signaling pathway that targets clathrin-mediated endocytosis at the plasma membrane.

RESULTS

Auxin Inhibits PIN Internalization by a Rapid, Nontranscriptional Mechanism

PIN proteins dynamically cycle between the endosomes and the plasma membrane (Geldner et al., 2001; Dhonukshe et al., 2007). Plasma membrane-localized PIN1 rapidly internalizes in response to the vesicle trafficking inhibitor brefeldin A (BFA) (Geldner et al., 2001), and this intracellular PIN accumulation is inhibited by auxins (Paciorek et al., 2005). In addition, auxin mediates with slower kinetics the degradation of PIN proteins (Sieberer et al., 2000; Abas et al., 2006). The auxin effects on PIN internalization and PIN degradation involve distinct mechanisms (Sieberer et al., 2000; Paciorek et al., 2005; Abas et al., 2006). These processes can be largely distinguished by BFA treatments at 25 and 50 μM that inhibit preferentially recycling or also vacuolar targeting for degradation, respectively (Sieberer et al., 2000; Abas et al., 2006; Kleine-Vehn et al., 2008).

We addressed the characteristics of the auxin signaling mechanism for inhibiting PIN internalization. It is experimentally established that the auxin regulation based on nuclear signaling requires at least ~10–15 min for execution (Badescu and Napier, 2006), whereas auxin inhibited the PIN2-GFP internalization more rapidly (<5 min) (Figures 1A and 1B). This suggests that this process does not involve auxin-dependent regulation of gene expression. Consistently, chemical inhibition of transcription (cordycepine or actinomycin D treatment) (Figure S1 available online) or de novo protein synthesis (cycloheximide treatment) (Figure 1C) does not prevent the auxin-mediated inhibition of PIN internalization.

Auxin Inhibits PIN Internalization by a TIR1-Independent Pathway

To elucidate the molecular mechanism by which auxin inhibits PIN internalization, we first tested the involvement of the TIR1-mediated signaling by genetical or chemical interference with different steps of this pathway. We analyzed (1) the quadruple *tir1/afb* mutant deficient in most of the TIR1/AFB auxin receptors function, (2) dominant lines conditionally expressing the stabilized transcriptional inhibitor IAA17 (*HS::axr3-1*), (3) stabilized mutations in other Aux/IAA-encoding genes (*axr2-1*, *axr3-1*, *shy2-2*, and *slr-1*), and (4) silenced lines for multiple ARFs *(2X35S::miRNA160)*, as well as (5) seedlings treated with the proteasome inhibitor MG132 that interferes with auxin-mediated degradation of Aux/IAA repressors (Figures 1D–1H and Figure S1). These manipulations have all been shown to strongly inhibit TIR1-mediated transcriptional auxin responses (Timpte et al., 1994; Fukaki et al., 2002; Tian et al., 2002; Knox et al., 2003; Dharmasiri et al., 2005b). Moreover, interference with the TIR1 pathway can be visualized (Figures 1I–1M and Figure S1) by monitoring the activity of the synthetic auxin-responsive promoter DR5, which is an indicator for TIR1-dependent gene