Polar auxin transport together with local synthesis and turnover are crucial for establishing auxin gradients, which determine an array of plant developmental pathways. Transcription factor-mediated control of the genes involved in these processes is steadily receiving increased attention, including efforts to find regulators determining the expression of major auxin efflux carriers of the PIN family. Now, Kong et al. (2017) have provided evidence of a direct link between auxin metabolism and transport mediated by PINs that is controlled by the transcription factor WRINKLED1. In its major form as indole-3-acetic acid (IAA), the plant hormone auxin drives plant growth and development and controls fundamental cellular processes, such as division, expansion and differentiation. Hence, transport of auxin plays a pivotal role in nearly all aspects of plant development, and efflux carriers of the PIN-FORMED (PIN) family have been described as key components exerting this role. Numerous studies have shown that the polar localization of PINs is a critical vectorial feature of auxin flow in Arabidopsis (Zazimalova et al., 2014). Many older studies had shown hormonal and environmental cues to be major regulators of their expression (Vieten et al., 2005; see also review by Krecek et al., 2009). However, the identification of molecular components determining PIN expression levels turned out to be very difficult, and the first detailed molecular mechanisms and protein factors acting upstream of these genes have only been uncovered relatively recently. Even less is known about how PINs are co-regulated with other auxin-relevant targets.

Regulation of PINs

Among the first factors shown to regulate PIN expression was the MADS transcription factor XAANTAL2 (XAL2), also known as AGAMOUS-LIKE 14 (AGL14) (Box 1). It was shown that XAL2, which otherwise regulates meristem proliferation and flowering transition, is required for expression of PIN4 and PIN1 (Tapia-López et al., 2008; Garay-Arroyo et al., 2013). Meristem defects in the xal2 mutant resemble those seen in pin4 and/or pin1 knockouts or in their higher order mutant combination, and xal2 mutants also show reduced free IAA levels and polar auxin transport (Friml et al., 2002; Bilou et al., 2005; Garay-Arroyo et al., 2013).

Another transcription factor controlling PIN expression, PPP1 (PIN2 PROMOTER BINDING PROTEIN 1), is a...
Box 1. Summary of evidence for the direct regulation of PIN expression by different transcription factors

| Transcriptional regulator | Promoter target | Method | Notes | Reference |
|---------------------------|-----------------|--------|-------|-----------|
| XAL2 (AGL14) | PIN4, PIN1 | ChIP-qPCR | | Garay-Arroyo et al. (2013) |
| BRM | PIN1, PIN2, PIN3, PIN4 and PIN7 | ChIP-qPCR | Mediates chromatin association with PIN loci | Yang et al. (2015) |
| PPP1 | PIN2, PIN1 | Y1H screen, EMSA | | Benjamins et al. (2016) |
| CRF2, 3, 7 | PIN7 | Y1H screen, ChIP-qPCR, transient co-expression in vivo | | Simaskova et al. (2015) |
| ARF7 | PIN3 | Y1H assay, ChIP-qPCR | Also binds FLP | Chen et al. (2015) |
| FLP (MYB124), MYB88 | PIN3, PIN7 | Y1H assay, ChIP-qPCR, EMSA | Also binds YUC5 and TAA1 loci for auxin synthesis | Wang et al. (2015) |
| IDD16 | PIN1 | ChIP-qPCR | | Cui et al. (2013) |
| WRI1 | PIN4, PIN5 | EMSA | Also binds to GH3.3 (auxin conjugation) | Kong et al. (2017) |

It has been known for a long time that PIN expression can be modulated rapidly by exogenously applied auxins (Vieten et al., 2005). Using the chromatin immunoprecipitation (ChIP) assay technique, both Chen et al. (2015) and Wang et al. (2015) found that the widely studied AUXIN RESPONSE FACTOR 7 (ARF7) in concert with the MYB transcription factor FOUR LIPS (FLP, MYB124), and partially with FLP parologue MYB88, directly regulates expression of closely related PIN3 and PIN7. Moreover, FLP is itself a direct target of ARF7. Accordingly, genetic and biochemical approaches, supported by mathematical modelling, revealed that both ARF7 and FLP are required for PIN3-mediated lateral root development (Chen et al., 2015; Wang et al., 2015).

Naturally, transcriptional regulators that, besides PINs, also regulate other genes involved in auxin-dependent processes are important. However, only one example of such regulation has been provided to date. INDETERMINATE DOMAIN (IDD) transcription factors belong to the plant-specific family of developmentally important transcriptional regulators (Cui et al., 2013; Long et al., 2015; Yang et al., 2015). IDD14, IDD15 and IDD16 are, among other processes, required for inflorescence and silique formation and their (ortho)gravitropic responses. It has been reported that IDD16 and possibly IDD14 bind to the promoters of PIN1 and of genes required for auxin synthesis, namely TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS (YUC) 5 and TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS (YUC) 1. Consequently, idd multiple mutants show several auxin-related defects, including altered levels of free IAA and moderately reduced ability to transport auxin (Cui et al., 2013).
WRINKLED1 (WR1) regulation

IAA conjugation is a highly important auxin deactivation process and, in contrast to auxin synthesis, our knowledge of this pathway is relatively limited. It is known that most of the total plant IAA pools are present as low molecular weight IAA conjugates with sugars or amino acids (Ludwig-Müller, 2011). Several members of the GRETCHEN HAGEN 3 (GH3) gene family encode auxin-inducible acyl amido synthetases required for IAA conjugation with amino acids (Staswick et al., 2005). Free IAA can be released back from some of the IAA conjugates by the action of IAA-amido hydrolases. Although GH3 genes are classically associated with early auxin transcriptional responses (Hagen and Guilfoyle, 2002), no direct upstream regulator of their expression had been identified until now.

Kong and co-authors have now identified WRINKLED1 (WR1) as a possible upstream regulator, coupling both auxin conjugation and transport (Kong et al., 2017). This AP2 transcription factor (a class AP2 ANT) is required for controlling fatty acid and oil synthesis (Cernac and Benning, 2004). The present study reveals that WR1 binds to the promoter of GH3.3 in electrophoretic mobility shift assays (EMSAs). Among other GH3 transcripts, expression of GH3.3 genes is particularly elevated in the wri1-1 mutant. This is accompanied by higher content of the IAA-Asp conjugate, while the levels of free IAA remain unchanged. Interestingly, the authors also show that WRI1, besides a non-canonical WRI1-binding motif in the GH3.3 promoter, also binds to promoters of PIN4 and PIN5 (but not to PIN1 and PIN6 in their experimental setup). Consequently, the expression of several PIN genes (PIN1, PIN3, PIN5 and PIN6) is reduced in the wri1-1 background. In agreement with these data, sensitivity to exogenously applied auxin and polar auxin transport are also affected in the wri1-1 mutant (Kong et al., 2017).

Several auxin transport facilitators, including the subclass of so-called short PINs (PIN5, 6 and 8), reside at the endoplasmic reticulum (ER) (Mravec et al., 2009; Barbez et al., 2012). Overexpression of PIN5 in BY-2 cells leads to increased levels of IAA-Asp and IAA-Glu (products of irreversible conjugation; Östing et al., 1998; Kowalczyk and Sandberg, 2001) at the expense of free IAA. It was proposed that PIN5 may enhance the transport of IAA from the cytoplasm to the ER, which might impede intercellular IAA transport by ER-located auxin degradation (Mravec et al., 2009; Simon et al., 2016). Importantly, Kong et al. observed up-regulation of GH3.3 expression but a drop in PIN5 expression in wri1-1 mutants. This suggests a possible link between the activity of GH3.3 and (PIN-mediated) intracellular auxin compartmentalization. In line with this, several IAA-amino acid hydrolases were recently shown to localize to the ER (Sanchez Carranza et al., 2016). The shared transcriptional dependency of both PIN5 and PIN6 with GH3.3 (Kong et al., 2017) leads to speculation as to whether conveying auxin into the ER might enhance the rapidity of specific, irreversible auxin deactivation in this regulatory pathway in a WRI1-dependent manner. Moreover, although the role of fatty acid synthesis has been proposed to interfere with auxin transport, this largely concerned polarity and subcellular trafficking dynamics of PINs (Roudier et al., 2010; Markham et al., 2011). Thus, the link between transcriptional regulation of PIN expression and fatty acid synthesis would be an interesting topic for future research.

Acknowledgements

Supported by the Czech Science Foundation (P501/12/0934 and GA16-26428S, to K.R.) and the Ministry of Education, Youth and Sports of the Czech Republic (the National Program for Sustainability, NPUII-LQ1601).

Note Added in Proof

While this article was in press, Simonini et al. (2017) revealed, using genome wide approaches, that also ARF3 protein, among others, directly regulates expression of PINs along with the genes required for auxin synthesis.

Key words: Arabidopsis, endoplasmatic reticulum, GH3, IAA conjugation, PIN expression, polar auxin transport, transcriptional regulation.

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