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REVIEW PAPER

Multiple links between shade avoidance and auxin networks

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Abstract

Auxin has emerged as a key player in the adjustment of plant morphology to the challenge imposed by variable environmental conditions. Shade-avoidance responses, including the promotion of stem and petiole growth, leaf hyponasty, and the inhibition of branching, involve an intimate connection between light and auxin signalling. Low activity of photo-sensory receptors caused by the presence of neighbouring vegetation enhances the activity of PHYTOCHROME INTERACTING FACTORs (PIFs), which directly promote the expression of genes involved in auxin biosynthesis, conjugation, transport, perception, and signalling. In seedlings, neighbour signals increase auxin levels in the foliage, which then moves to the stem, where it reaches epidermal tissues to promote growth. However, this model only partially accounts for shade-avoidance responses (which may also occur in the absence of increased auxin levels), and understanding the whole picture will require further insight into the functional significance of the multiple links between shade and auxin networks.

Key words: Auxin, cryptochrome, growth, phytochrome, PHYTOCHROME INTERACTING FACTOR (PIF), shade avoidance.

Introduction

Light and auxin control growth

The formation of the basic body of the plant depends strongly on the information (space, time, context) about the internal environment that auxin provides to the cells. Although each species has distinctive features of its body pattern, and different species tend to colonize land spaces where their pattern maximizes fitness, the environment is strongly variable and therefore the plants have to face stressful conditions. In response to this scenario, plants have evolved mechanisms to monitor the environment and dynamically adjust their body form and function. Plants can be extraordinarily plastic in response to environmental changes. For instance, plants perceive light cues from the environment thanks to the action of a battery of photo-sensory receptors, connected to signalling networks that mediate morphological changes as well as developmental transitions through their life cycle (Xu *et al.*, 2015; Su *et al.*, 2017).

Only a few decades ago, it was reasonable to question whether photo-sensory receptors had their own specific molecular pathways to control growth or whether they actually impinged upon hormone signalling. Now it is clear that changes in the action of plant hormones are involved in every morphological and developmental process modulated by light. Actually, auxin has emerged as a key player in the adaptive response to multiple stresses (Wolters and Jürgens, 2009). In this review, we focus on the regulation of auxin metabolism,

transport, and perception in response to changes in the light environment caused by the presence of neighbouring vegetation and the impact of this regulation on plant growth.

Some of the most conspicuous responses to the light signals produced by neighbouring vegetation are the promotion of stem and petiole growth, and leaf hyponasty (more erect position) (Franklin, 2008; Casal, 2013; Fraser *et al.*, 2016; Ballaré and Pierik, 2017). Leaves can also turn their direction of growth on the horizontal plane, away from the position of their close neighbours. Light signals produced by neighbours can also reduce leaf lamina growth and branching (growth of buds in the axil of leaves). All these changes are called shade-avoidance responses because they tend to reduce the extent of current or future shade by placing the leaves at higher strata within the canopy and reducing the growth of organs (e.g. branches) that become shaded at the base of the canopy. Some shade-avoidance responses such as enhanced growth and reduced branching are mimicked by the application of exogenous auxin treatments (Zhao *et al.*, 2002; Chapman *et al.*, 2012; Reddy *et al.*, 2014; de Wit *et al.*, 2015) (Fig. 1) and in mutants with enhanced levels of auxin (King *et al.*, 1995; Delarue *et al.*, 1998; Zhao *et al.*, 2001). Particularly striking is the tight correlation between the diurnal fluctuations in sensitivity of hypocotyl growth to brief periods of shade or auxin treatment, which suggests shared underlying mechanisms involved in the control of the responses to both stimuli (Sellaro *et al.*, 2012).

Exactly 30 years ago, Child and Smith (1987) noted the analogy between the complex fine kinetics of stem growth in response to low red/far-red ratios (simulating the presence of neighbour plants) in light-grown mustard plants and the response of stem and coleoptile sections to the addition of auxin. At that time, they concluded that due to the scarce mechanistic information 'it would be premature to assume that the far-red light effect in mustard operates through auxin' (Child and Smith, 1987). The current review demonstrates how significantly our knowledge has advanced since those pioneer observations. We focus on shade signals, auxin, and growth, whereas for a wider view of selected cases involving different light signals, hormones, and physiological processes we recommend the excellent recent review by de Wit *et al.* (2016*a*). Our review takes an auxin-centric view of the shade-avoidance response; however, it is well established that other hormones are also essential in the control of plant responses to light conditions, often tightly connected to auxin (Vanstraelen and Benková, 2012).

Overview of light signalling during shade avoidance

The presence of neighbouring vegetation modifies the light environment experienced by plants (Franklin, 2008; Casal, 2013; Fraser *et al.*, 2016; Ballaré and Pierik, 2017). Green leaves strongly absorb photosynthetically active radiation (400–700 nm, including blue and red light) but reflect and transmit far-red light (700–800 nm) much more efficiently. Nearby neighbours can reflect far-red light and thereby lower the red/far-red ratio even without infringing shade. If neighbours are sufficiently large (tall) and close, they will shade other plants, reducing not only the red/far-red ratio but also the absolute red and blue irradiance. These changes, collectively called here neighbour signals, are perceived by plants mainly due to the reduction in the activities of the red/far-red photo-sensory receptor phytochrome B (phyB) and the blue light photo-sensory receptors cryptochromes 1 (cry1) and cry2 that they impose.

Active phyB binds PHYTOCHROME INTERACTING FACTOR 1 (PIF1), PIF3, PIF4, PIF5, and PIF7, which are basic helix–loop–helix (bHLH) transcription factors (Leivar and Quail, 2011). As a result of this, phyB reduces the activity of PIFs by facilitating their phosphorylation and degradation by the 26S proteasome and/or reducing their capacity to bind their DNA targets (Leivar and Quail, 2011; Li *et al.*, 2012). Therefore, when plants are exposed to neighbour signals, which reduce phyB activity, PIFs enhance their impact on the transcriptome as they tend to promote the expression of a large number of direct targets (Hornitschek *et al.*, 2012; Leivar *et al.*, 2012*b*; Li *et al.*, 2012). The *pif7*, *pif4*, *pif5*, and *pif3* mutations reduce plant responses to neighbour signals (Lorrain *et al.*, 2008; Leivar *et al.*, 2012*a*; Li *et al.*, 2012; Sellaro *et al.*, 2012). PIF4 and PIF5 are also bound by cry1 and cry2, and low levels of blue light increase the abundance of these PIFs (Pedmale *et al.*, 2016).

The reduced red and blue light under shade also favours the nuclear accumulation of CONSTITUTIVE

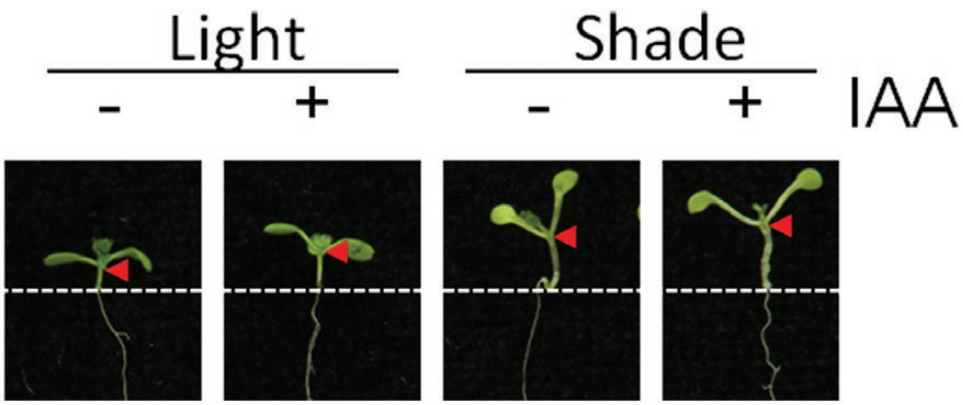

Fig. 1. Addition of auxin mimics growth responses induced by shade. Images of aerial parts of 7-day-old seedlings of *A. thaliana* captured after 48 h of addition of 1 μM indole acetic acid (IAA), simulated shade (Pacín *et al.*, 2013), or the combination of both treatments.

PHOTOMORPHOGENIC 1 (COP1) (Pacín *et al.*, 2013). COP1 is part of complexes with E3 ligase activity, which target nuclear proteins for degradation (Lau and Deng, 2012). In response to shade of increasing duration, COP1 favours the degradation of LONG HYPOCOTYL IN FAR-RED LIGHT (HFR1) (Pacín *et al.*, 2016). HFR1 forms heterodimers at least with PIF4 and PIF5, which are unable to bind DNA (Hornitschek *et al.*, 2009). Therefore, COP1 reinforces the activity of PIFs in the presence of neighbour signals by inducing HFR1 degradation (Pacín *et al.*, 2016).

Overview of auxin signalling

Natural auxin, indole acetic acid, is synthesized from tryptophan in the cytosol by multiple pathways. The best characterized route combines the sequential action of the TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS 1 (TAA1) and YUCCA (YUC) flavin monooxygenase enzymes, which mediate tryptophan conversion to indole-3-pyruvate (IPA) and then to auxin, respectively (reviewed by Ljung, 2013). YUC enzymes appear to be rate limiting (Won *et al.*, 2011). The Gretchen Hagen3 (GH3) family of amido synthases together with the action of UDP-glucose transferases UGT84B1, UGT74E2, and UGT74D1 conjugate auxin, mainly to sugars and amino acids (Jackson *et al.*, 2001; Tognetti *et al.*, 2010; Jin *et al.*, 2013), and this process is reversed by amido-hydrolases INDOLE-ACETIC ACID LEUCINE RESISTANT 1 (ILR1) and ILR1-like (ILL), which hydrolyse the conjugates, restoring free auxin (Woodward and Bartel, 2005; Ludwig-Müller, 2011). In addition, auxin oxidation by DIOXYGENASE FOR AUXIN OXIDATION 1 (DAO1) and DAO2 has been described recently as the main auxin catabolic process in *Arabidopsis thaliana* (Mellor *et al.*, 2016; Wang *et al.*, 2016). Therefore, free (active) auxin homeostasis depends on synthesis, auxin conjugation, hydrolysis of auxin conjugates, and auxin degradation.

In addition, free auxin levels also depend on transport from or to other parts of the plant. At the low pH of the apoplast, auxin becomes protonated and can enter the cell by diffusion. In specific cell types, auxin is also transported into the cytosol by auxin influx carrier proteins, such as the AUXIN RESISTANT1/LIKE AUXIN RESISTANT (AUX1/LAX) family (Swarup and Péret, 2012). Inside the cell, auxin becomes negatively charged and therefore requires specific efflux carriers such as PIN-FORMED (PIN) and ATP-BINDING-CASSETE B TYPE (ABCB) to be transported across the cell membrane to the apoplast (Zazímalová *et al.*, 2010). A less characterized group of transport proteins, the PIN-LIKES (PILS), could be responsible for the intracellular transport of auxin between the cytosol and the endoplasmic reticulum (Barbez *et al.*, 2012).

Auxin regulates gene expression through direct physical interaction with the TRANSPORT INHIBITOR RESPONSE 1/AUXIN SIGNALING F-BOX (TIR1/AFBs) nuclear proteins. TIR1/AFBs are auxin receptors and constitute the F-box subunits of the SKP1–CULLIN–F BOX (SCF)-type E3 ligase, $SCF^{TIR1\text{-}AFBs}$. Auxin binding to $SCF^{TIR1\text{-}AFBs}$ in the nucleus results in the targeted ubiquitination and degradation of the AUXIN/INDOLE-3-ACETIC ACID (Aux/IAA) co-receptor (Dharmasiri *et al.*, 2005*a*, *b*; Kepinski and Leyser, 2005; Tan *et al.*, 2007; Calderón Villalobos *et al.*, 2012). Aux/IAAs are short-lived proteins that function as active repressors by forming dimers with AUXIN RESPONSE FACTORs (ARFs) (Gray *et al.*, 2001). Aux/IAA degradation relieves the inhibition on ARF transcription factors, allowing the modulation of auxin response genes and the consequent promotion, or in a few cases repression, of their expression.

The early, or primary auxin response genes, are components of three major families: *Aux/IAA* genes, *SMALL AUXIN UP RNA* (*SAUR*) genes, and *GH3* genes (Hagen and Guilfoyle, 2002). Enhanced expression of the genes encoding Aux/IAA and GH3 proteins add a negative feedback loop that would be important to limit the auxin signal (Salehin *et al.*, 2015; Mellor *et al.*, 2016). SAURs are a large family of 79 small proteins unique to plants with no obvious motifs suggestive of a biochemical function. Recently, SAUR-dependent promotion of elongation growth was associated with the activation of the plasma membrane H^+-ATPase by inhibiting different phosphatases (Fendrych *et al.*, 2016; Spartz *et al.*, 2017).

Auxin modulates the expression of a large number of transcription factors (including members of the HD-Zip superfamily, AP2/EREBP-type, zinc finger-like, and MYB-like zinc finger-like transcription factors), implicated in development and hormone crosstalk (Chapman and Estelle, 2009). Auxin also induces genes related to cell expansion such as xyloglucan endotransglycosylase/hydrolase (*XTH*) genes, expansins, and β-glucanases (Kotake *et al.*, 2000; Goda *et al.*, 2004).

Shade-avoidance responses require auxin

Shade-avoidance responses are impaired in mutants affected in auxin biosynthesis, conjugation, transport, perception, or downstream signalling (Table 1), and also as a result of pharmacological treatments that influence auxin transport or perception (Keuskamp *et al.*, 2010, 2011; de Wit *et al.*, 2015). These observations indicate that shade-avoidance responses require the whole chain of auxin-related events. Therefore, the alternative possibility that light/shade signalling simply recruited (in evolution) selected auxin-related components, which then gained an auxin-independent function, can be ruled out. Since shade-avoidance responses require growth, a trivial interpretation would be that without auxin signalling there is no growth and, hence, shade-avoidance responses. While the latter is true, the subsequent sections describe that there is a much more intimate signalling connection between the pathways downstream of photo-sensory receptors and auxin.

PIFs connect neighbour signals to multiple auxin-related genes

We generated a list of genes grouped in 13 families according to their different auxin-related functions (synthesis,

conjugation, hydrolysis of conjugates, degradation, transport, perception, and signalling; Supplementary Table S1 at *JXB* online). Twelve of these 13 families have members with promoters bound by PIFs (Fig. 2). The only exception corresponds to the genes involved in auxin oxidation. Approximately 45% of these genes are bound by PIF4, 20% by PIF5, and 14% by PIF3 ($P<0.0001$; $P<0.0001$; $P=0.068$ when compared with the 17, 5, and 15% of bound genes in the whole genome, respectively). In total, 53% of the auxin-related genes are binding targets of PIF4, PIF5, and/or PIF3. Conversely, only 22% of the auxin response genes (i.e. genes that respond to the addition of auxin, Goda *et al.*, 2008) are binding targets of PIF4, 7% of PIF5, and 5% of PIF3 (which is not significantly different from genomic values). Although

Table 1. *Auxin mutants impaired in shade-avoidance responses*

Auxin process	Mutant	References
Biosynthesis	*sav3*, *yuc2 yuc4*, *yuc2 yuc3 yuc5 yuc9*	Vandenbussche *et al.* (2003); Tao *et al.* (2008); Moreno *et al.* (2009); Keuskamp *et al.* (2010); Crepy and Casal (2015); Kohnen *et al.* (2016)
Conjugation	*gh3.17*	Zheng *et al.* (2016)
Transport	*pin3*, *pin3 pin7*, *pin3 pin4 pin7*, *abcb1*, *abcb19*	Pierik *et al.* (2009); Keuskamp *et al.* (2010, 2011); Ge *et al.* (2017)
Perception	*tir1*, *tir1 afb1 afb2 afb3*, *axr1*[a]	Steindler *et al.* (1999); Pierik *et al.* (2009); Finlayson *et al.* (2010); Keuskamp *et al.* (2010, 2011)
Signalling	*axr2/iaa7*[b], *msg2/iaa19*[b], *axr3/iaa17*[b]	Pierik *et al.* (2009); Sellaro *et al.* (2011); Procko *et al.* (2016)

[a]The *axr1* mutant is affected in the normal regulation of TIR1/AFB activity (Gray *et al.*, 2001)
[b]Gain-of-function mutant.

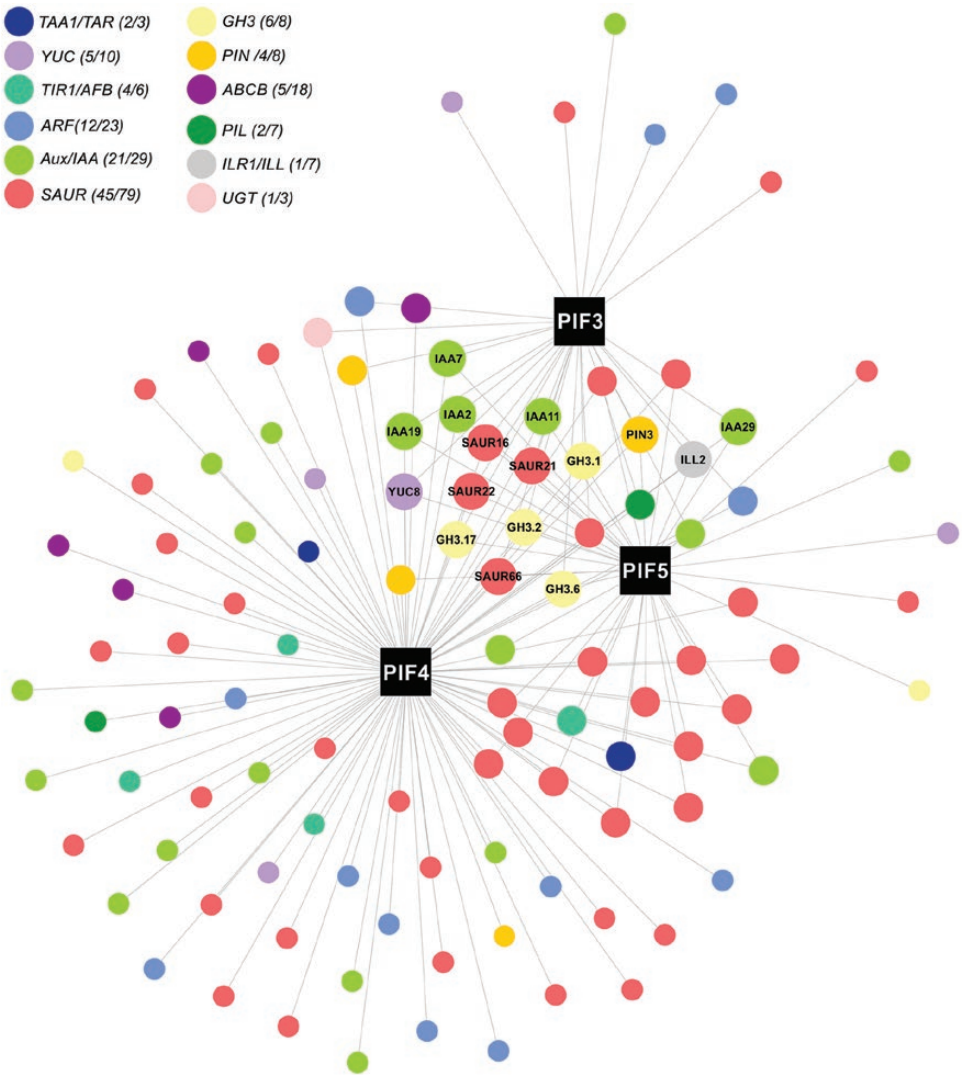

Fig. 2. PIF3, PIF4, and/or PIF5 bind multiple genes involved in auxin biosynthesis, conjugation, transport, perception, and signalling. The interaction network shows PIF3, PIF4, and PIF5 target genes identified by ChIP-seq analyses (Hornitschek *et al.*, 2012; Oh *et al.*, 2012; Zhang *et al.*, 2013).

there are no global ChIP analyses yet, PIF7 also interacts with *YUC5*, *YUC8*, and *YUC9* promoters (Li *et al.*, 2012). Of course, this information alone is not enough to conclude that all these genes are direct transcriptional targets of PIFs during shade avoidance, but the observations presented in subsequent sections do confirm this to be the case at least for some genes.

Neighbour light signals modify the expression of auxin-related genes

The tight wiring between light/shade signalling and auxin signalling via PIFs is consistent with the large proportion of genes involved in auxin functions showing expression responses to neighbour light signals. Several studies have identified auxin-related genes over-represented among those genes promoted by low red/far-red ratios (Devlin *et al.*, 2003; Sessa *et al.*, 2005; Leivar *et al.*, 2012*b*; Li *et al.*, 2012; Kohnen *et al.*, 2016), simulated shade (Pacín *et al.*, 2016), natural shade (Sellaro *et al.*, 2011), and a pulse of far-red light to reduce phyB activity at the end of the day (Nito *et al.*, 2015).

We pay particular attention to expression patterns because natural variation in the response of auxin-related genes to neighbour signals appears to be important for shade-avoidance responses (Bush *et al.*, 2015). The domesticated tomato *Solanum lycopersicum* cultivar M82 shows attenuated shade-avoidance responses compared with the wild species *Solanum pennellii*. Introgression lines resulting from crosses between these two species show large variation in the magnitude of shade-avoidance responses. The lines with reduced expression of auxin-related genes under shade displayed reduced stem growth responses (Bush *et al.*, 2015).

Figure 3 provides an overview of the shade-induced modifications in the transcript levels of the 13 gene families responsible for different auxin-related functions (Supplementary Table S1) (Sellaro *et al.*, 2011; Pacín *et al.*, 2016). We present both the median and dispersion of the whole family and the pattern of expression of one of its members. These analyses provide complementary information because the description at family level suggests that some functions are consistently affected by shade signals. The presence of multiple members in one gene family provides a way of differential regulation by different signals, but actually eight of these families show a general trend of response to shade (*TAA1*/*TAR*, *GH3*, *ILR1*/*ILL*, *DAO*, *PILS*, *TIR*/*AFB*, *AUX*/*IAA*, and *SAUR*). In addition, two families show no general trend but include members with a significant response (*YUC* and *ABCB*), and three families are largely independent of shade at the whole-seedling level (*IAA-UGT*, *PIN*, and *ARF*). For comparative purposes, in Supplementary Fig. S1 we show the expression patterns of these families during de-etiolation (i.e. when the seedling experiences its first exposure to light after overtopping the soil) (Peschke and Kretsch, 2011) and in Supplementary Fig. S2 we show the effects of long days compared with short days (representative of different seasons) (Mockler *et al.*, 2007). The changes appear to be more extensive during shade avoidance (eight families show a general trend) than during de-etiolation (four families), or in response to day length (three families).

Auxin biosynthesis genes

The median expression of the *TAA1*/*TAR* family is reduced by neighbour signals, and *TAR2* provides a good example of this pattern (Fig. 3). Conversely, *YUC2*, *YUC3*, *YUC5*, *YUC8*, and *YUC9* expression can be rapidly induced by shade signals in young seedlings (Won *et al.*, 2011; Brandt *et al.*, 2012; Li *et al.*, 2012; Nito *et al.*, 2015; Kohnen *et al.*, 2016; Müller-Moulé *et al.*, 2016) (Fig. 3). However, other *YUC* genes can reduce their expression (*YUC4*, *YUC6*, and *YUC7*), depending on the context (Nito *et al.*, 2015; Kohnen *et al.*, 2016), and the median of expression of all *YUC* is largely unaffected.

Auxin conjugation and catabolism genes

The expression of the *GH3* gene family involved in auxin conjugation shows an overall negative trend with light because it is enhanced by shade compared with light (Fig.3), short compared with long days (Supplementary Fig. S2), and darkness compared with light during de-etiolation (Supplementary Fig. S3). Multiple members of this family (*GH3.2*, *GH3.3*, *GH3.6*, *GH3.5*, and *GH3.17*) are induced by shade signals (Li *et al.*, 2012; Procko *et al.*, 2014; Nito *et al.*, 2015). In contrast to the *GH3* family, UDP-glucose transferases, also involved in auxin conjugation, do not respond to shade (Fig. 3). Five of the six members of the amido-hydrolase family (*ILR1*, *ILL3*, *ILL4*, *ILL5*, and *ILL6*) show enhanced expression in response to natural shade (Fig. 3). Neighbour signals enhance *DAO1* and *DAO2* expression (Fig. 3). However, shorter exposures to neighbour signals can reduce *DAO2* expression (Kohnen *et al.*, 2016), indicating that light effects on these genes are strongly context dependent.

Auxin transport genes

The overall median expression of the *PIN* family is not affected by relatively prolonged shade (Fig. 3). However, specific members (e.g. *PIN3*, *PIN4*, and *PIN7*) do transiently increase their expression in response to neighbour light signals (Keuskamp *et al.*, 2010; Kohnen *et al.*, 2016). The *PILS* family shows an overall positive trend in response to shade, which is particularly strong for *PILS3* and *PILS5* (Fig. 3). In addition, *ABCB19* expression is promoted by shade (Fig. 3).

Auxin perception and signalling genes

TIR1/*AFB* genes show a general promotion by shade signals (Fig. 3). The effect is significant for *AFB1* but *TIR1*, *AFB2*, and *AFB3* show a similar trend. Despite the fact that several *ARF* genes are binding targets of PIFs (Fig. 2), these genes do not show consistent regulation by shade signals (Fig. 3) or by light/dark conditions (Supplementary Figs S1, S2). The picture is substantially different for *Aux*/*IAA* genes and *SAUR* genes that are early auxin response genes, and show enhanced expression in response to shade (Leivar *et al.*, 2012*b*; Li *et al.*,

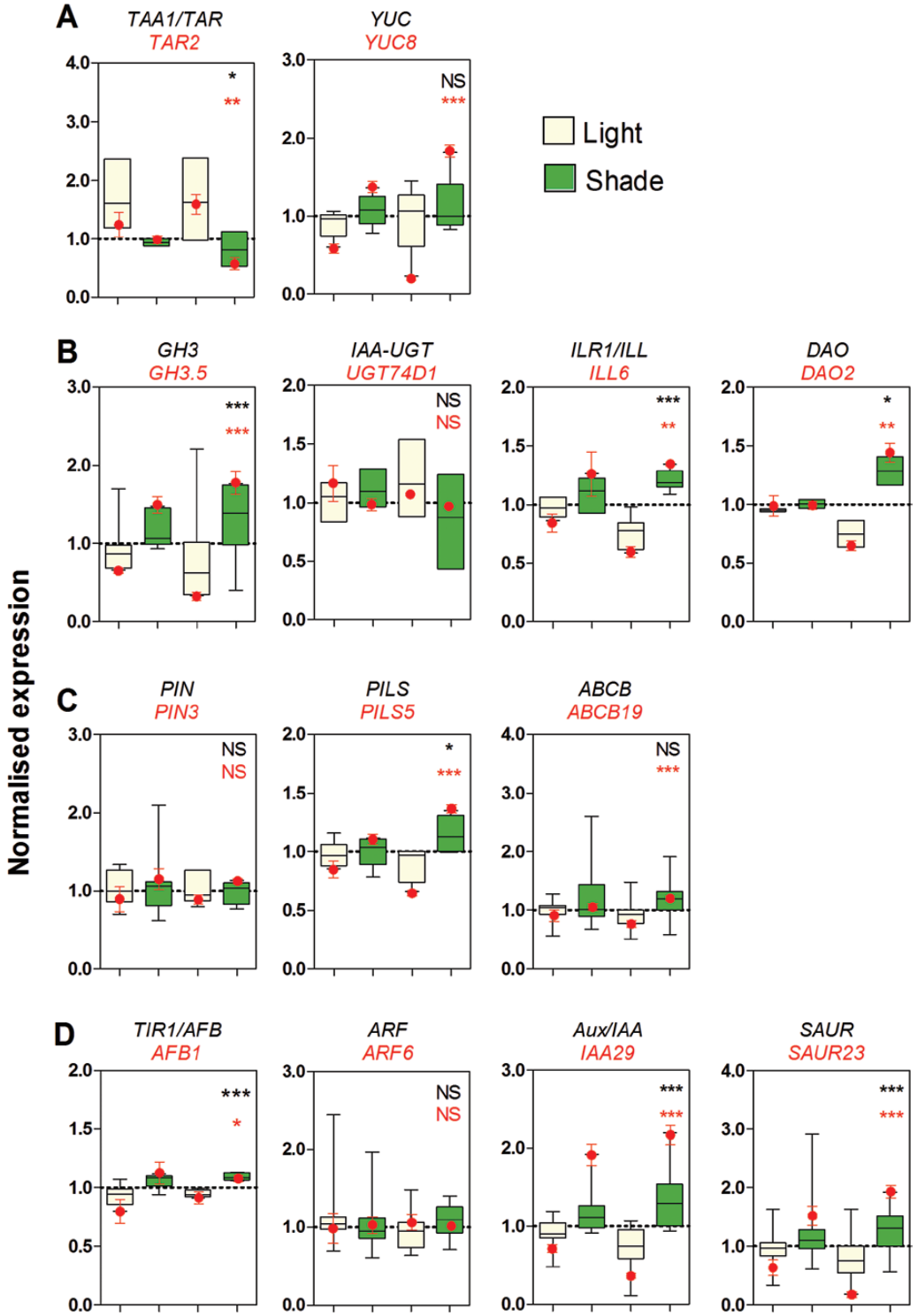

Fig. 3. Shade signals control the expression of genes involved in auxin biosynthesis (A), conjugation–degradation (B), transport (C), and perception and signalling (D). Gene expression was normalized to the median of each gene for each experiment. For each gene family, box plots show the median, 1–3 interquartile range, and the 95% confidence interval of normalized values to identify whole family trends. In addition, circles show the expression (±SE) of a relevant family gene member. Left columns: *A. thaliana* plants grown under white light photoperiods of 10 h for 3 d were transferred to simulated shade conditions 1 h after the beginning of the photoperiod, or left as a control under white light and harvested 6 h later (drawn after Pacín *et al.*, 2016). Right columns: *A. thaliana* plants grown under sunlight or natural shade under photoperiods of 10 h were harvested 9 h after the beginning of the photoperiod of the third day of treatment (drawn after Sellaro *et al.*, 2011). The significance of the effect of shade in two-way ANOVA is indicated: *P<0.05; **P<0.01; ***P<0.001.

2012; Kohnen *et al.*, 2016) (Fig. 3). The regulation of these genes by neighbour signals is extensive. For instance, 23 of the 79 members of the *SAUR* family, and 12 of the 29 members of the *Aux/IAA* family are induced by natural shade compared with sunlight (Sellaro *et al.*, 2011). Some of the most conspicuous responsive genes are *IAA2*, *IAA7*, *IAA19*, *IAA29*,

and multiple members of the *SAUR19* (*SAUR19–SAUR24*) and *SAUR63* (*SAUR61–SAUR68* and *SAUR75*) subfamilies. Many of these genes also enhance their expression in dark- compared with light-grown seedlings (Supplementary Fig. S1), and in short- compared with long-day-grown seedlings (Supplementary Fig. S2).

Auxin response genes

The previous paragraphs show that many genes involved in the control of the cellular levels of active auxin (biosynthesis, degradation, conjugation, and transport; Supplementary Table S1) and its perception and signalling respond to neighbour signals. Auxin itself modifies gene expression and therefore it is valid to ask whether neighbour signals systematically modify the expression of auxin-responsive genes. The genes that respond to auxin include many related to growth such as *XTR*/*XTH*, expansins, extensins, and *Class III peroxidases*, as well as others involved in the homeostasis of active auxin levels and auxin signalling, such as *Aux*/*IAA*, *SAUR*, and *GH3* genes. The meta-analysis of a set of auxin-responsive genes shows that there is an overall tendency of auxin-induced genes to increase their expression in response to neighbour signals and of auxin-repressed genes to reduce their expression in response to neighbour signals (Sellaro *et al.*, 2017). However, this pattern is context specific and, although no cases of inverted patterns have been observed, in some contexts auxin response genes fail to show a shift in expression as a result of neighbour signals. The correlation between auxin and neighbour light signals is stronger for auxin-induced than for auxin-repressed genes (Sellaro *et al.*, 2017).

Kinetics and localization of changes induced by shade signals

Figure 4 describes the early kinetics (0–3 h) of the changes in gene expression of selected auxin-related genes in the cotyledons and in the hypocotyl of *A. thaliana* seedlings transferred from high to low red/far-red ratios typical of the presence of neighbouring vegetation. The analysis is based on a detailed data set published recently by the Fankhauser group (Kohnen *et al.*, 2016). The changes in expression are fast, with a lag of no more than 15 min, at least in one of the two organs. In the cotyledons, most of these genes have already achieved their maximum response 90 min after the beginning of low red/far-red ratios.

Some genes such as *PIN3*, *IAA29*, *ABCB19*, and *SAUR23* increase their expression similarly in cotyledons and hypocotyls. *DAO2* decreases its expression similarly in both organs (Fig. 4). However, other genes show differences in the responses of hypocotyl and cotyledons. For instance, *YUC8* and *TAA1* have a longer lag phase in the hypocotyl. The expression of *YUC8* increases in the cotyledons between 0 min and 90 min of neighbour signals, when it reaches a peak. In the hypocotyl, the lag is of ~90 min, and *YUC8* expression increases sharply between 90 min and 180 min. The decrease in *TAA1* expression has a lag of 15 min in the cotyledons and 45 min in the hypocotyl. Other genes only respond in one of the two organs; for example, *YUC9* is only induced in the cotyledons. Conversely, *GH3.1* and *GH3.5* are induced by neighbour signals in both the cotyledon and hypocotyls, but with higher intensity in the hypocotyl, while *YUC3*, *ILL6*, and *AFB1* are induced specifically in the hypocotyl (Kohnen *et al.*, 2016). Finally, other genes have opposite responses in both organs: *PILS5* is induced by neighbour signals in the hypocotyl and repressed in the cotyledons, whereas *YUC2* is rapidly induced in the cotyledons and transiently inhibited in the hypocotyl (Kohnen *et al.*, 2016).

To provide an overview of the potential functional implications of the changes in gene expression, we calculated the median fold change of gene groups involved in the control of free auxin levels (genes with both positive and negative impact), auxin transport, auxin perception, and early response to auxin (Fig. 4B). Shade signals promote the expression of genes with both positive and negative effects on free auxin, however with a stronger impact on the first group. This would be consistent with a positive balance between synthesis, conjugation, and degradation (actually the genes involved in degradation decrease their expression in response to neighbour signals), particularly early after the beginning of neighbour signals in the cotyledons. There is also a positive effect of neighbour signals on the expression of genes involved in auxin transport and auxin perception, the latter mainly in the hypocotyl. Early auxin response genes provide a proxy for auxin signalling status. These genes increase their expression almost simultaneously in both organs, but with a more robust tendency in the hypocotyl (Fig. 4B).

By looking at these data, it is possible to predict a rapid increase in free auxin levels in the cotyledons and the transport of auxin from the cotyledons to the hypocotyl, where the increased auxin levels, together with the higher sensitivity provided by the stronger abundance of receptors, would enhance the expression of early response genes. The enhanced auxin signalling in the hypocotyl would be involved in its growth promotion in response to neighbour signals. At a later stage, there might be a contribution of enhanced auxin synthesis in the hypocotyl itself. This sequence of events is only hypothetical because there are important control steps between gene mRNA levels and activity of the proteins that execute the relevant functions. However, some of these predictions are already confirmed in the available literature (see below).

The response patterns can be strongly context dependent. For instance, in the cotyledons, 11 *SAUR* genes increase their expression when dark-grown seedlings are exposed to white light to increase photo-receptor activity (Sun *et al.*, 2016), but also when light-grown seedlings are transferred from high to low red/far-red ratios to reduce photo-receptor activity (Kohnen *et al.*, 2016). Conversely, in the hypocotyl, 27 *SAUR* genes reduce their expression when dark-grown seedlings are exposed to white light (Sun *et al.*, 2016) and increase their expression when light-grown seedlings are transferred from high to low red/far-red ratios (Kohnen *et al.*, 2016), reversibly following photo-receptor status.

The expression changes observed in a given organ are not necessarily the sole consequence of the neighbour signal perceived in that organ. Rather, interorgan signalling appears to

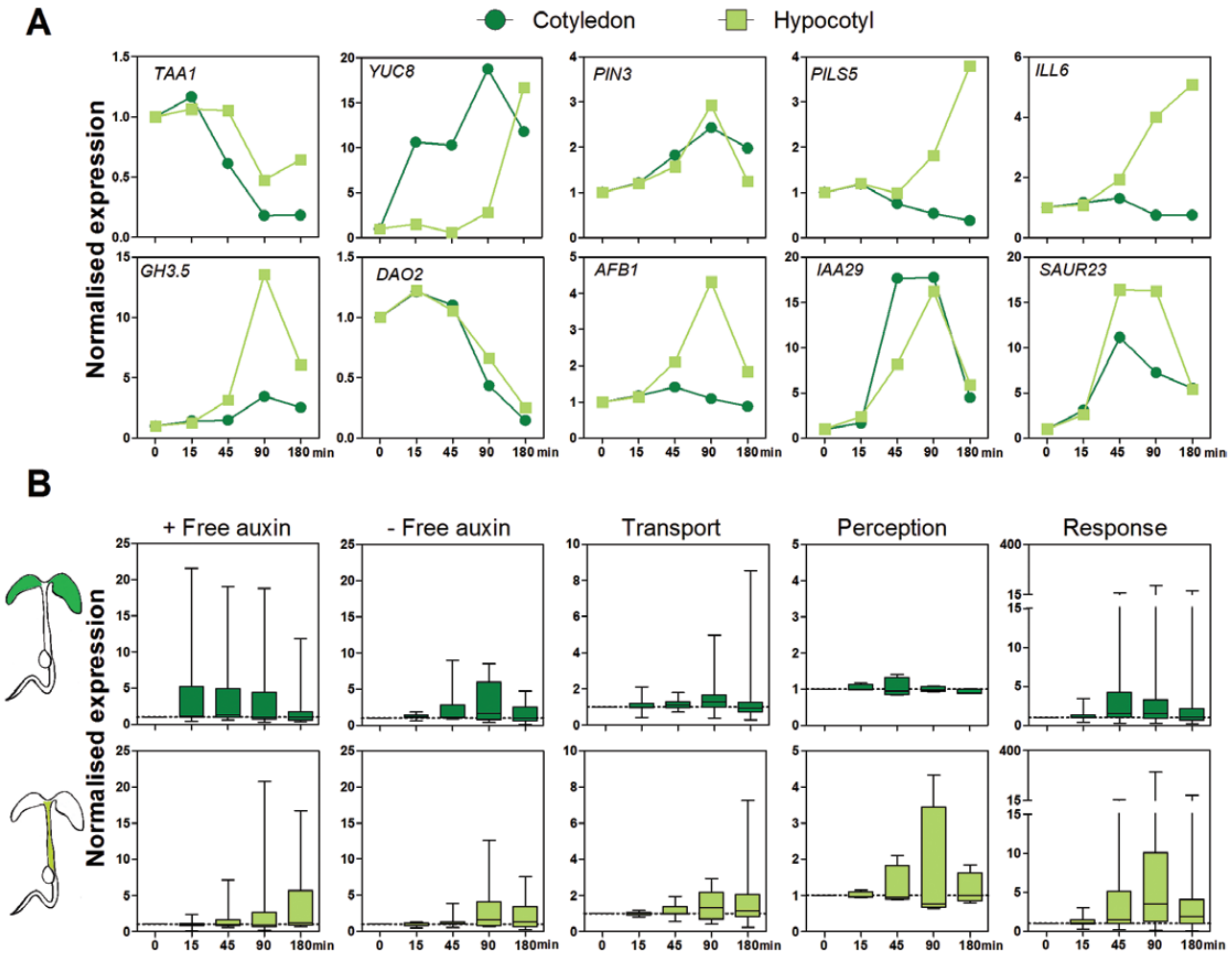

Fig. 4. Auxin-related gene expression shows different patterns of temporal and organ regulation by shade signals. (A) Time course of expression (relative to white light at time 0) of selected auxin-related genes in cotyledon and hypocotyls of *A. thaliana* in response to a shift from high to low red/far-red ratios of the white light (data from Kohnen *et al.*, 2016). (B) Time course of expression of functional gene groups. Genes that increase (+, synthesis, reversion of conjugation) or decrease (–, conjugation, degradation) free auxin levels, and genes involved in auxin transport, auxin perception (receptors), and early response to auxin (*Aux/IAA*, *GH3*, and *SAUR*) in cotyledon and hypocotyl. For each functional group of genes, box plots show the median, 1–3 interquartile range, and the 95% confidence interval of relative expression values (data from Kohnen *et al.*, 2016).

be important. A recent study compared the transcriptome responses to a pulse of far-red light given at the end of the day to reduce phyB activity in the shoot apex (which contained the meristem, basal parts of leaf primordia, and short fragments of vasculature) and cotyledons (Nito *et al.*, 2015). The promotion of expression of auxin-responsive genes was prominent in the apex. Selective organ irradiation with far-red light indicated that *IAA19*, *IAA29*, *SAUR22*, and *YUC3* are more strongly promoted in the shoot apex than in the cotyledons by treatments applied to the cotyledons. Furthermore, treatments applied to the apex have a weaker effect. This pattern suggests that there is a signal moving from the cotyledon to stimulate auxin-responsive genes in the apex. Since this response is impaired in the *sav3* mutant, the signal might be auxin itself. The opposite pattern was observed for *YUC2*, which was induced in the cotyledons by far-red light given to the apex, suggesting the participation of a shoot apex–cotyledon retrograde signal, which is unlikely to be auxin dependent because the pattern persists in the *sav3* mutant (Nito *et al.*, 2015). Finally, other genes such as *YUC9* showed an autonomous response and increased their expression in the cotyledons when far-red light was given to the cotyledons. In support of local responses in the hypocotyl, independent from cotyledon-driven auxin, a robust induction of primary responsive gene expression is still detected in *sav3*, *pin3 pin4 pin7*, and *yuc2 yuc5 yuc8 yuc9* (Hornitschek *et al.*, 2012; Bou-Torrent *et al.*, 2014; Kohnen *et al.*, 2016).

Despite differential background expression of some of the genes involved in auxin biosynthesis, conjugation–degradation, transport, perception, and signalling in the whole hypocotyl compared with the epidermis of *Brassica rapa* seedlings (Procko *et al.*, 2016), the relative impact of low, compared with high, red/far-red ratios is very similar (Supplementary Fig. S4).

Gene expression responses to neighbour signals depend on PIFs

To quantify the relationship between promoter binding by PIFs and the gene expression response to shade, we calculated

the simulated shade/light fold change for the genes involved in free auxin homeostasis, transport, perception, and signalling that showed significant responses to shade in the wild type (Sellaro *et al.*, 2011; Pacín *et al.*, 2016). For the genes with promoters bound by PIF3, PIF4, and/or PIF5, the fold change was clearly >1 in the wild type but not in the *pifq* mutant, indicating that in these genes PIFs cause the promotion of gene expression induced by simulated shade (Fig. 5). Conversely, for the genes with promoters not bound by PIF3, PIF4, and/or PIF5, the fold change was only marginally above 1 in the wild type (Fig. 5). In other words, all the auxin-related genes that showed significant promotion by simulated shade in the wild type failed to respond in the *pifq* mutant (Pacín *et al.*, 2016) and 73% are known to be bound by PIF3, PIF4, and/or PIF5. Therefore, PIF binding to the gene promoters has a fundamental role in the auxin-related transcriptome in response to shade.

Of particular relevance is the observation that in ChIP experiments PIF3, PIF4, PIF5, and PIF7 directly bind the promoters of *YUC3*, *YUC5*, *YUC6*, *YUC8*, and *YUC9* genes (Hornitschek *et al.*, 2012; Li *et al.*, 2012; Pfeiffer *et al.*, 2014), and the expression of these genes fails to respond to shade in the *pif4 pif5* and *pif7* mutants (Hornitschek *et al.*, 2012; Li *et al.*, 2012), which can therefore be defined as direct targets of PIFs. The *cop1* mutation, which reduces the activity of PIFs, impairs the promotion of *YUC* genes by shade (Pacín *et al.*, 2016).

Beyond transcription: neighbour signals modify free auxin, Aux/IAA, and PIN dynamics

Neighbour signals increase free auxin levels

Neighbour signals induce the rapid (1–2 h) accumulation of auxin in the cotyledons of *A. thaliana* and *B. rapa* seedlings (Tao *et al.*, 2008; Procko *et al.*, 2014), which are the primary organs of perception of neighbour signals in these species (Procko *et al.*, 2014). At a later stage, higher levels of auxin become detectable in the hypocotyl of sunflower (Kurepin *et al.*, 2007) or *A. thaliana* (Keuskamp *et al.*, 2010) seedlings. The accumulation of auxin is the consequence of the enhanced activity of PIFs in the presence of neighbour signals. PIFs bind to and increase the activity of the promoters of selected members of the *YUC* gene family in the cotyledons (*YUC9* and *YUC2*). Consistently with the role of PIFs (and the positive regulator COP1) and the TAA–YUC pathway, *pif* (multiple), *cop1*, *yuc* (multiple), and *sav3* mutants fail to increase auxin levels in response to neighbour signals (Won *et al.*, 2011; Hornitschek *et al.*, 2012; Li *et al.*, 2012; Pacín *et al.*, 2016) and both *pif* (Hornitschek *et al.*, 2012; Li *et al.*, 2012; de Wit *et al.*, 2015) and *cop1* (Pacín *et al.*, 2016) mutants fail to increase *YUC* expression.

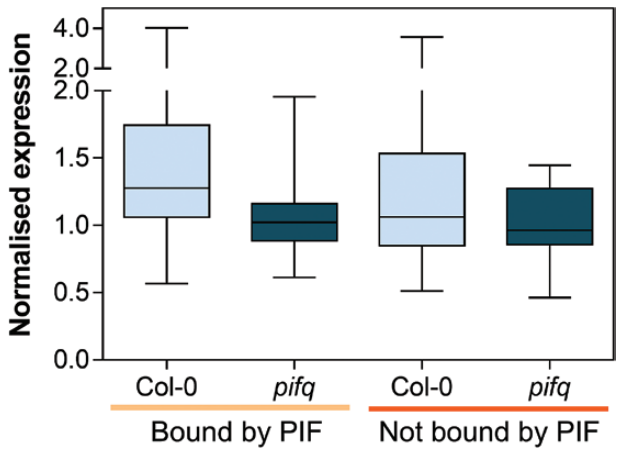

Fig. 5. The response of auxin-related genes to neighbour signals depends on PIFs. The fold change in expression was calculated for the genes involved in free auxin homeostasis, transport, perception and signalling (Supplementary Table S1), which showed significant responses to simulated shade compared with white light in the wild type of *A. thaliana* (Pacín *et al.*, 2016). Box plots show the median, 1–3 interquartile range, and the 95% confidence interval of the fold change of the genes that are either bound by PIFs or not bound by PIFs in the wild type (Col-0) or the *pifq* (*pif1 pif3 pif4 pif5*) mutant.

In the stem, the indole acetic acid amido synthetase GH3.17/VAS2 catalyses the formation of auxin conjugates with glutamate, destined for degradation (Zheng *et al.*, 2016). Other members of the GH3 family produce reversible conjugates with indole acetic acid. The *gh3.17* mutant seedlings show enhanced levels of free auxin and stronger *DR5:GUS* staining in the hypocotyl. The expression of *GH3.17* in hypocotyls declines modestly after 1 h of shade treatment, and more appreciably after 24 h in the shade. This response to neighbour signals could contribute to the shade-induced accumulation of free auxin in the hypocotyl (Zheng *et al.*, 2016).

Since neighbour signals increase auxin levels, part of the changes in transcriptome induced by these signals might be the indirect consequence of more auxin. This mechanism could account for the enhanced expression of auxin-promoted genes and reduced expression of auxin-repressed genes in young seedlings exposed to shade (Sellaro *et al.*, 2017). However, some effects are likely to be the direct consequence of the many additional points of direct action of PIFs on auxin-related genes. For instance, auxin-responsive gene expression is enhanced in the mature stem segments of the *phyB* mutant (which exhibits constitutive shade avoidance) despite its low auxin levels, suggesting the modulation of auxin signalling independently of auxin abundance and transport (Krishna Reddy and Finlayson, 2014).

Neighbour signals reduce the stability of Aux/IAA

Tian *et al.* (2003) have detected no effects of light on the turnover of IAA3 on extracts from etiolated *A. thaliana* seedlings. However, since shade signals can increase auxin levels, and auxin induces via TIR1/AFB receptors the ubiquitination of Aux/IAA, targeting them to degradation in the 26S proteasome (Dharmasiri *et al.*, 2005*a*, *b*; Kepinski and Leyser, 2005; Tan *et al.*, 2007; Calderón Villalobos *et al.*, 2012), shade signals should reduce the stability of Aux/IAA. To determine these effects quantitatively, we have implemented an experimental set-up in plant cells, utilizing a genetically encoded quantitative biosensor to monitor the stability of Aux/IAA proteins under different shade regimes (Wend *et al.*, 2013;

Winkler *et al.*, 2017). Figure 6 describes the kinetics of IAA19 in *A. thaliana* protoplasts exposed to simulated shade. As predicted, increasing durations of the shade signal decrease the abundance of IAA19. The kinetics of a DELLA protein (RGA) are shown for comparative purposes because DELLA proteins have already been shown to decrease their abundance in response to neighbour signals (Djakovic-Petrovic *et al.*, 2007). Although the decreased abundance of IAA19 is probably caused by increased auxin under simulated shade, a more direct control by phytochromes cannot be ruled out, particularly because both phyA (Colón-Carmona *et al.*, 2000) and phyB (Tian *et al.*, 2003) physically interact with several Aux/IAAs.

Neighbour signals modify PIN abundance and localization

The reduced accumulation of auxin and *DR5:GUS* reporter activity in the hypocotyl of seedlings treated with the auxin transport inhibitor NPA supports the view that auxin produced in the cotyledon in response to neighbour signals is at least partially transported to the hypocotyl (Tao *et al.*, 2008; Keuskamp *et al.*, 2010). The enhanced expression of auxin transporter genes by neighbour signals (Figs 3, 4) would serve this purpose. In addition, neighbour signals promote the re-localization of PIN3–green fluorescent protein (GFP) fusion protein from the basal end to the lateral side of the endodermal cells, and this could promote auxin transport to the epidermis (Keuskamp *et al.*, 2010), which has a key role in limiting hypocotyl elongation. The reduced PIN1 protein level in the hypocotyls in response to shade might help to retain auxin in the stem by mitigating its transport to the root (Sassi *et al.*, 2013).

Differences and similarities between the impact of neighbour signals perceived by phyB and cry

Compared with sunlight, vegetation shade reduces the activity of phyB, cry1, and cry2. The reduction in phyB and cryptochrome activities can be simulated independently. For instance, supplementing white light with far-red light lowers the red/far-red ratio and phyB activity without affecting cryptochromes. Conversely, lowering the amount of blue light by filtering white light with selective (yellow-orange) filters reduces cryptochrome activity without affecting phyB. Low red/far-red and low blue light can *per se* induce shade-avoidance responses such as the promotion of stem growth.

Mutations of auxin-related genes can affect the promotion of hypocotyl or petiole growth induced either by lowering the red/far-red ratio (Tao *et al.*, 2008; Keuskamp *et al.*, 2010; Sasidharan *et al.*, 2010; Cole *et al.*, 2011) or by blue light (Keller *et al.*, 2011; Keuskamp *et al.*, 2011). However, some of the mutants that are effective in impairing petiole growth and hyponastic responses to low red/far-red (Tao *et al.*, 2008;

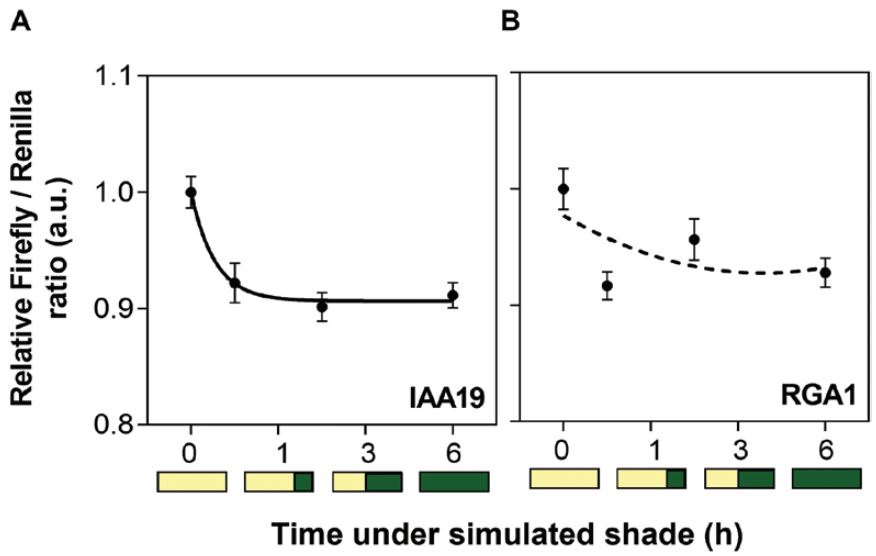

Fig 6. Quantitative effects of low red/far-red ratios on auxin signalling. (A) *Arabidopsis thaliana* protoplasts were transiently transformed with a genetically encoded auxin biosensor as a proxy of IAA19 stability. At 24 h after transfection, the protoplasts were exposed for 6 h to white light or simulated shade (low red/far-red ratio), or for 5 h or 3 h to white light followed by simulated shade, respectively, for 1 h or 3 h (see schemes in abscissae). White light corresponds to a mixture of red light (1.00 μmol m^{-2} s^{-1}) and blue light (0.56 μmol m^{-2} s^{-1}). Simulated shade included red and blue light (same irradiances as under white light) plus supplementary far-red light (10.00 μmol m^{-2} s^{-1}). Each waveband was provided by light-emitting diodes. The ratiometric sensor construct consists of the cDNA of *IAA19* fused to firefly luciferase (sensor module, SM). Renilla luciferase is fused to the SM and separated by a 2A peptide, leading to equimolar expression of the SM and renilla as described (Wend *et al.*, 2013; Winkler *et al.*, 2017). Renilla and firefly luminescence was determined after 6 h of treatment as described (Wend *et al.*, 2013). The SM is degraded upon increased auxin levels. Renilla luciferase levels are insensitive to the treatment and thus used as a normalization element. Depicted is the firefly/renilla ratio (set at 1 for the white light control); a lower ratio indicates higher degradation/reduced stability of IAA19. (B). As in (A), but the result of a sensor construct for RGA is shown for comparison. Data are means (±SE) of six biological replicates. The sensor constructs were engineered in the pBS/pGEN016 vector backbone by AQUA cloning (Beyer *et al.*, 2015); protoplast preparation and luminescence determinations were as described (Wend *et al.*, 2013; Ochoa-Fernandez *et al.*, 2016). A similar construct lacking the sensor module (Ctrl-Luc) was used as negative control (Wend *et al.*, 2013) (see Supplementary Fig. S3).

Sasidharan *et al.*, 2010) have no effect on these responses to blue light (Keller *et al.*, 2011). Furthermore, low red/far-red ratios increase free auxin abundance in the cotyledons (Tao *et al.*, 2008) but low blue light does not detectably affect auxin levels (Pedmale *et al.*, 2016). These observations suggest that the physiological responses to low red/far-red ratios, which are normally more intense than those to lowering blue light, are also more limited by auxin signalling and require increased auxin levels.

Pedmale *et al.* (2016) found that auxin-regulated genes are not over-represented in the low blue light transcriptome, despite the fact that this feature is typical in the case of low red/far-red ratios (Hornitschek *et al.*, 2012; Leivar *et al.*, 2012*b*; Li *et al.*, 2012). They propose that PIF4 and PIF5 control of hypocotyl elongation in response to blue light involves predominantly the regulation of cell wall-modifying proteins. In Fig. 7, we plot the log-transformed ratios of expression of auxin-related genes in seedlings exposed either to low red/far-red ratios (left) or to low blue light (right) against the expression ratio observed in seedlings exposed to simulated shade (which combines low red/far-red, low red, and low blue); where in all cases the white light controls were used to calculate the ratios. Despite a number of differences in the experimental protocols of two data sets coming from different laboratories, there is a very strong correlation between the effects of lowering the red/far-red ratio and simulating shade (Fig. 7A). As expected, simulated shade has a stronger response because it entails a stronger signal. Although in the case of the low blue light response there is more dispersion (lower *R* values), the correlation with the effects of simulated shade is still very significant (Fig. 7B). These results indicate that cryptochromes make a contribution to the changes in expression of auxin-related genes caused by shade. For instance, low blue light promotes the expression of *IAA7*, *IAA19*, *IAA29*, *GH3.5*, *PILS5*, and *SAUR19* (Pedmale *et al.*, 2016). The expression of *IAA19* is more strongly promoted when low red/far-red and low blue signals are combined (de Wit *et al.*, 2016*b*), indicating that auxin-associated gene expression is proportionally correlated to the intensity of shade.

The reduction of both phyB and cryptochrome activities by shade signals increases the abundance of PIFs (de Wit *et al.*, 2016*b*) and the nuclear abundance of COP1 (Pacín *et al.*, 2013), but these effects are larger in response to the light conditions that modify phyB status. Therefore, the observed differences in the ability to modify the expression of auxin-related genes and enhance auxin abundance could be the result of a more intense shade signal transduced by phyB than through cryptochromes, which would differentially affect processes requiring a signal threshold. However, qualitative differences between the signalling networks of phyB and cryptochromes could also be involved.

Other photo-sensory receptors are also involved in the control of auxin signalling. UV-B perceived by UVR8 strongly inhibits the induction of *YUC8*, *YUC9*, and *IAA29* expression by low red/far-red ratios (Hayes *et al.*, 2014). The long-term integral of irradiance of white light perceived by phyA elevates the auxin signalling status (described by DR5:GUS staining), making the plants grown under high light more sensitive to neighbour signals perceived by phyB (Trupkin *et al.*, 2014).

Auxin in plant responses to neighbour signals

Auxin is at the core of the current model of the mechanisms involved in the promotion of hypocotyl growth by neighbour signals in *A. thaliana* (Fig. 8A). The low red/far-red ratios of shade, compared with sunlight, reduce the activity of phyB, and enhance the abundance of PIF7, PIF4, PIF5, and PIF3. The perception of the red/far-red cue takes places in the cotyledons, where PIFs bind the promoter of *YUC* genes and enhance auxin levels. Auxin is transported from the cotyledons to the hypocotyl by PINs and ABCBs. In the hypocotyl, PIN3 is reoriented from the basal to the lateral side of the cell membrane and drives the auxin flux to the epidermis, which is the growth-limiting tissue (Fig. 8). Low red/far-red

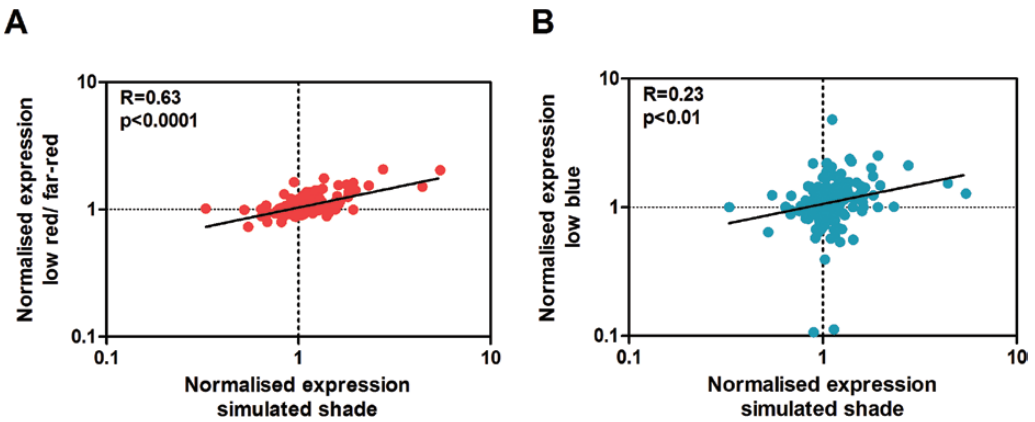

Fig. 7. Contribution of phytochrome and cryptochrome to the control of auxin-related gene expression by simulated shade in *A. thaliana*. (A) Low/high red/far-red ratio log-transformed ratios of expression of auxin-related genes (calculated after Leivar *et al.*, 2012*b*) plotted against the simulated shade/white light log-transformed ratios of expression of the same genes (calculated after Pacín *et al.*, 2016). (B) Low/high blue light log-transformed ratios of expression of auxin-related genes (calculated after Pedmale *et al.*, 2016) plotted against the simulated shade/white light log-transformed ratios of expression of the same genes (calculated after Pacín *et al.*, 2016). Each point represents the log expression ratio of one gene. The coefficient of determination and the significance of the correlation are indicated.

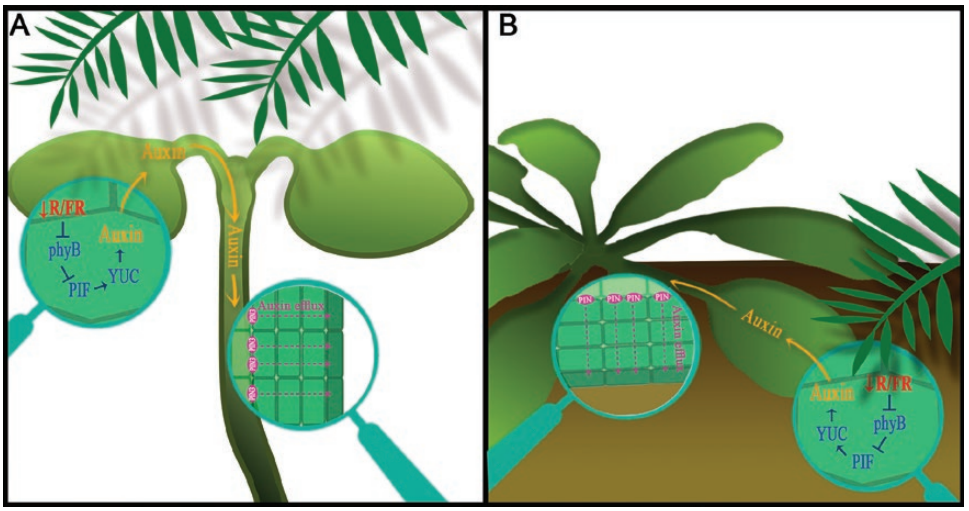

Fig. 8. Model depicting the contribution of auxin to the promotion of hypocotyl growth (A) and leaf hyponasty (B) by neighbour signals in *A. thaliana*.

ratios reaching the hypocotyl would provide a local reinforcement of the growth promotion by lowering the expression of *GH3.17* involved in a type of auxin conjugation that is followed by degradation. This model is supported genetically, because mutations at any of these steps (in particular those involving auxin, Table 1) distort the shade-avoidance response. Furthermore, induction of *YUC3* expression specifically in cotyledons is enough to trigger hypocotyl elongation in seedlings grown in the absence of neighbour signals, indicating that cotyledon-driven auxin is enough to promote stem growth (Kohnen *et al.*, 2016). In addition, the expression of either a bacterial auxin biosynthesis gene or the auxin-inactivating enzyme GH3.17/VAS2 in the epidermis triggers enhanced and decreased hypocotyl elongation in shade, respectively (Procko *et al.*, 2016). In the epidermis, auxin would act in part by enhancing brassinosteroid signalling, probably by increasing the synthesis of this hormone (Procko *et al.*, 2016). Key features of this model could apply for the leaf lamina as a source of auxin for enhanced petiole growth in response to a low red/far-red ratio (de Wit *et al.*, 2015). The shift from low to high red/far-red ratios also reduces the growth of the branches emerging from the buds present in the axil of the uppermost leaves of the rosette of *A. thaliana*, and these buds show a reduction in auxin levels (Reddy *et al.*, 2013), providing another example of growth induced by neighbour signals and related to changes in auxin levels. The reduced blue light levels of shade would enhance these responses (de Wit *et al.*, 2016*b*; Pedmale *et al.*, 2016) (Fig. 7).

The model based on auxin driven to the stem from the cotyledons (Fig. 8) also applies to the promotion by low red/far-red of the phototropic response to horizontal gradients of blue light (Goyal *et al.*, 2016). The reduced activity of phyB caused by low red/far-red ratios enhances the activity of PIFs, which are necessary and sufficient to support phototropism. PIFs modulate the phototropic response by promoting the expression of *YUC2*, *YUC5*, *YUC8*, and *YUC9*, which are necessary for bending in light-grown seedlings. Therefore, shade would increase auxin available to generate the hormone gradient that drives hypocotyl bending (Goyal *et al.*, 2016). This scenario is very different from that observed in etiolated seedlings, where multiple *yuc* or multiple *pif* mutants do not impair the phototropic response, suggesting that auxin is limiting for the phototropic response in green but not in etiolated seedlings.

The analogy with the model involving light signal perception in the cotyledon followed by auxin-mediated hypocotyl growth promotion can also be extended to leaf hyponasty (upwards leaf movement) in response to neighbour light signals (Michaud *et al.*, 2017; Pantazopoulou *et al.*, 2017). Leaf hyponasty involves faster growth of the lower than the upper side of the petiole to elevate the leaf lamina, and is a typical shade-avoidance response of *A. thaliana* rosettes. However, the low red/far-red ratio signal has to be perceived by the lamina, and treatment of the petiole alone is not effective. In the lamina tip, low red/far-red ratios reduce phyB activity, and increase the activity of PIFs, the expression of *YUC* genes, and the synthesis of auxin. Then, auxin would be transported to the petiole and distributed asymmetrically between its abaxial and adaxial sides to promote bending in a process involving PINs. The petiole is able to perceive low red/far-red to promote elongation but not to promote hyponasty, and hyponasty can be induced by auxin applied to the lamina tip, but not to the petiole itself. This spatial separation of perception and response would optimize the ability to react to the early warning signals of neighbours (Michaud *et al.*, 2017; Pantazopoulou *et al.*, 2017).

Although the above model involving changes in auxin levels is well established, it cannot explain all shade-avoidance responses. In 7-day-old seedlings (advanced hypocotyl growth stage) and in the petioles and lamina of 15-day-old seedlings (rosette stage), the levels of auxin increase rapidly but transiently in response to low red/far-red ratios, as high auxin levels are no longer detectable 24 h after the beginning of the neighbour signals (Bou-Torrent *et al.*, 2014; de Wit *et al.*, 2015). Lowering the red/far-red ratio immediately before starting the night promotes the growth of the petiole and enhances the expression of auxin response genes without causing detectable changes in the levels of auxin in the petiole

or in the leaf blade (Kozuka *et al.*, 2010). In tomato seedlings, low red/far-red ratios enhance the growth of the stem and cause a rapid and persistent promotion of expression of selected auxin response genes (*IAA* and *SAUR*), mainly in the stem, but no changes in auxin levels are detectable in the stem or leaves after 4 d of treatment (Cagnola *et al.*, 2012). Furthermore, changing from low to high red/far-red ratios reduces the levels of auxin in the basal main stem segments of *A. thaliana*, and this might contribute to rosette bud outgrowth, but the auxin response is transient and only slightly affects the expression of auxin-related genes (Holalu and Finlayson, 2017).

Under prolonged shade, the promotion of hypocotyl growth when auxin levels have returned to the pre-stimulation values might involve other hormones. For instance, in *A. thaliana* seedlings, gibberellins are elevated later than auxin (Bou-Torrent *et al.*, 2014). In the axillary buds of *A. thaliana* rosettes, abscisic acid is important to restrain growth (Reddy *et al.*, 2013; Holalu and Finlayson, 2017). However, persistent shade avoidance could also be mediated by some of the many points of action of PIFs on auxin-related processes beyond synthesis. Shade triggers the up-regulation of the *TIR1*/*AFB* gene family (Fig.3), and a rapid accumulation of AFB1 protein level in petioles, which might enhance the auxin sensitivity (de Wit *et al.*, 2015). PIF4 and PIF5 do not affect the early growth response to added auxin in the absence of neighbour signals (Chapman *et al.*, 2012); however, the scenario might be different under prolonged shade because lowering the red/far-red ratio and/or blue light enhances the abundance of PIFs. Actually, there is evidence in favour of enhanced sensitivity to auxin mediated by PIF4 and PIF5 under shade (Hersch *et al.*, 2014).

Conclusions

At the auxin network level, the reduction in the activity of photo-sensory receptors in response to light cues associated with neighbouring vegetation causes a concomitant increase in the activity of PIFs, which bind the promoters of genes involved in auxin metabolism, transport, perception, and signalling (Fig. 2). Many of these genes increase their expression, indicating that shade controls the auxin network at multiple points (Figs 3, 4). Beyond transcription, the information is fragmentary, but shade signals can also increase auxin levels and decrease the stability of Aux/IAA (Fig. 6). The promotion of stem growth caused by neighbour signals is impaired by mutations in auxin metabolism, transport, perception, and signalling genes (Table 1). This provides an example where an exogenous signal modulates growth by modifying the status of an endogenous signal at multiple points.

At the pathway level, a model has emerged (Fig. 8), where in the presence of neighbour signals transcriptional control of rate-limiting auxin synthesis genes enhances auxin levels in the cotyledons, which then travels to the hypocotyl to promote its growth (Fig. 1) and to facilitate its bending (phototropism). However, changes in auxin levels are transient and would be more important to overcome inertia than to sustain growth. The mechanisms of persistent shade-avoidance responses have not been established. They could involve increased sensitivity to auxin (de Wit *et al.*, 2015) as well as the participation of other hormones or factors.

Back to the network level, a higher sensitivity to auxin could be accounted for by some of the various links between PIFs and auxin-related process. In other words, future research should consider the multiple effects of neighbour signals on auxin-related genes for which the functional significance is not clear and, at the same time, on the apparent modifications in auxin sensitivity that have not been unequivocally related to specific actions of neighbour signals on the auxin-related molecular processes.

Supplementary data

Supplementary data are available at *JXB* online.

Table S1. List of auxin-related genes (auxin biosynthesis, conjugation–degradation, transport, perception, and signalling).

Fig. S1. De-etiolation signals control the expression of genes involved in auxin biosynthesis, conjugation–degradation, transport, perception, and signalling.

Fig. S2. Day-length controls the expression of genes involved in auxin biosynthesis, conjugation–degradation, transport, perception, and signalling.

Fig. S3. Negative control for the effects of low red/far-red ratios on auxin signalling.

Fig. S4. Similar impact of low red/far-red ratios on the expression of auxin-related genes in the epidermis and whole hypocotyl of *Brassica rapa*.

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