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# Meta-Analysis of the Transcriptome Reveals a Core Set of Shade-Avoidance Genes in Arabidopsis†

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## ABSTRACT

**The presence of neighboring vegetation modifies the light input perceived by photo-sensory receptors, initiating a signaling cascade that adjusts plant growth and physiology. Thousands of genes can change their expression during this process, but the structure of the transcriptional circuit is poorly understood. Here we present a meta-analysis of transcriptome data from *Arabidopsis thaliana* exposed to neighbor signals in different contexts, including organs where growth is promoted or inhibited by these signals. We identified a small set of genes that consistently and dynamically respond to neighbor light signals. This group is also affected by light during de-etiolation and day/night cycles. Among these genes, many of those with positive response to neighbor signals are binding targets of PHYTOCHROME-INTERACTING FACTORS (PIFs) and function as transcriptional regulators themselves, but none of these features is observed among those with negative response to neighbor signals. Changes. in neighbor signals can mimic the transcriptional signature of auxin, gibberellins, brassinosteroid, abscisic acid, ethylene, jasmonic acid and cytokinin but in a context-dependent manner. We propose the existence of a small core set of genes involved in downstream communication of PIF signaling status and in the control of light sensitivity and chloroplast metabolism.**

## INTRODUCTION

When plants are exposed to shade, the concomitant reduction in incoming photosynthetically active radiation may impose a severe limitation to the energy available for growth and survival. This has been the driving force for the evolution of sophisticated mechanisms that allow plant perception of signals of current or impending shade and the occurrence of growth and developmental responses that reduce the chances of continuing or becoming shaded (1). These processes are collectively called shade-avoidance responses.

Shade-avoidance responses are initiated by changes in the light environment caused by the presence of green neighboring vegetation (2). These cues include a reduction in the red/far-red ratio and red-light irradiance perceived by phytochrome B (phyB) and the reduction in blue light irradiance and blue/green ratio perceived by cryptochrome 1 (cry1) (3). Green leaves efficiently reflect far-red light, and therefore, the red/far-red ratio can be reduced by the presence of neighbors that do not infringe shade, providing a signal of nonshading neighbor proximity (4). Shade-avoidance responses include the promotion of stem and petiole growth, leaf hyponasty, reduced branching, reorientation of leaf or branch growth direction and acceleration of flowering (5–8). In addition, shade signals initiate tolerance responses (9). These acclimation responses do not reduce the probabilities of shade, but they can lower the energy expenditure under conditions where light for photosynthesis is scant, including reductions in the defense budget (10) and in the rate of stem respiration in plants grown under low red/far-red ratios (11).

Reduced phyB activity in response to shade enhances the abundance and/or target-DNA binding capacity of a set of the bHLH transcription factors PHYTOCHROME-INTERACTING FACTOR 3 (PIF3), PIF4, PIF5 and PIF7 (12–14). The activity of cry1 also affects PIF4 and PIF5 (15). In addition, reduced phyB and cry1 activity enhances the nuclear abundance of COP1 in the presence of neighbor signals (16), which in turn reduces the stability of LONG HYPOCOTYL IN FAR-RED (HFR1) (17) that is a negative regulator of PIFs (18). Thus, COP1 reinforces PIF activity. The PIFs bind a large set of genes, including those of rate limiting enzymes involved in auxin synthesis, and the higher levels of auxin promote stem growth (14,19).

The shade-avoidance syndrome is complex, and while some organs increase their growth in response to shade (stem, petioles), others show the opposite pattern (repressed buds, in some cases leaf lamina). After early work involving the 8K Affymetrix microarray (20), a number of studies have used the ATH1 microarray in different developmental contexts (young seedlings, leaf lamina, petioles, growing buds), different neighbor signals (simulated neighbor proximity, simulated shade, true canopy shade) and different directions of change (no neighbor to neighbor or vice versa) (Table 1) (17,19,21–27). Later, some studies have used RNAseq (19,28,29). There have been few attempts to characterize the structure of the transcriptional network. Leivar *et al.* (24) defined a set of genes that respond rapidly to low red/far-red in a PIF-dependent manner and comprises two subsets, one enriched in transcription factor genes and promoters containing G-box motifs, another lacking G-box motifs and enriched for auxin-responsive loci. By comparing transcriptome, they also identified a set of genes with expression repressed during de-etiolation and induced during shade avoidance, which contain G-box motifs (24).

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**Table 1.** Summary of the experiments used for the meta-analysis.

| Exp. | Refs | Organ(s) | Age (days) | Day (h)/Night (h) | White light | Neighbor signal | Treatment (h) |
|---|---|---|---|---|---|---|---|
| 1 | (21) | Seedling | 3 | 10/14 | Sunlight | Natural shade | 72 |
| 2 | (17) | Seedling | 3 | 10/14 | FL + FR* | FL + FR + GF | 9† |
| 3 | (22) | Seedling | 8 | 16/8 | FL | FL + FR | 1† |
| 4 | (23) | Seedling | 8 | 16/8 | FL | FL + FR | 24† |
| 5 | (24) | Seedling | 2 | 24/0 | FL | FL + FR | 3† |
| 6 | (27) | Seedling | 7 | 24/0 | FL | FL + FR | 1† |
| 7 | (19) | Seedling | 14 | 12/12 | FL | FL + FR | 2† |
| 8 | (25) | Leaf blade | 19 | 24/0 | FL | FL + EOD FR | 2† |
| 9 | (25) | Petiole | 19 | 24/0 | FL | FL + EOD FR | 2† |
| 10 | (26) | Bud | 3 (AP) | 18/6 | FL | FL + FR | 72‡ |
| 11 | (26) | Bud n-2 | 3 (AP) | 18/6 | FL | FL + FR | 72‡ |

Exp = experiment; Ref = reference; AP = after pollination; EOD = end-of-day pulse followed by darkness; FL = fluorescent white light; FR = far-red light; GF = green filter. *Red/Far-red ratio similar to sunlight. †The seedlings were grown under white light and transferred to the neighbor signal condition. ‡The seedlings were grown under neighbor signal conditions and transferred to white light.

The aim of this work was to investigate the structure of the transcriptional response to neighbor signals. To approach this issue, we conducted a meta-analysis of publically available and unpublished ATH1 microarray data. We define a core set of genes that consistently change their expression in response to neighbor signals despite differences in the developmental context.

## MATERIALS AND METHODS

*Selection of core- and context-specific genes.* For the meta-analysis, we used the expression datasets from ATH1 microarray experiments involving different neighbor signals described in Table 1. For each gene, expression data were normalized to the median of all light conditions and used for factorial ANOVA with neighbor signal and experiment as main factors. We first identified the genes showing significant effects of neighbor signals ($P < 0.011$, $q < 0.050$) (30). Then, these genes were divided into two groups. For this purpose, we calculated the ratio between the average corresponding to plants treated with neighbor signals and the average corresponding to the control. This ratio was calculated for each experiment and for the whole set of experiments. The genes that showed these ratios either >1 in all the cases or <1 in all the cases were defined as core genes (Table S1). The genes that failed to show all the ratios >1 or all the ratios <1 were defined as frequent responders, context-specific genes. Genes showing significant effects of the interaction among neighbor signals and experiment and no significant main effect of neighbor signals were classified as occasional responders, context-specific genes (Table S1).

*Function enrichment.* Genes coding transcription factors and chloroplast localized proteins were obtained from Arabidopsis Information Portal (https://www.araport.org/). Enrichment was calculated by comparing the total number of genes in each category to total genes represented in the ATH1 microarray. The statistical significance was calculated using chi-square tests with Yates correction or Fisher's exact test. Other enriched functions are based on Atecoecis (http://bioinformatics.psb.ugent.be/ATCOECIS/) (31).

*Hormone-responsive genes.* For each one of the hormones tested here, a set of positively and a set of negatively responsive genes were obtained from a previous selection (32). For each gene, expression data were normalized to the median calculated for all the experiments involving neighbor signals. For each experiment, control and neighbor signal conditions were compared by Student's *t*-test.

*Comparative responses to neighbor, de-etiolation and day/night signals.* To compare the gene expression responses to neighbor, de-etiolation and day/night signals, we used only experiments where the seedlings were at the hypocotyl stage. Therefore, experiments 1, 2 and 5 in Table 1 were selected to describe the responses to neighbor signals. Data from dark grown seedlings transferred for 4 h to white light or 1 h to red light compared to dark controls were used to investigate the response during de-etiolation (33,34). Seedlings harvested every 4 h throughout the 24-h cycle either under long days (16-h day/8-h night) or under short days (8-h day/16-h night) were used to investigate the day/night response (35). In addition to the core genes, we investigated a set of context-specific genes. For this purpose, normalized data of the three neighbor signal experiments were used for ANOVA and genes showing significant up-regulation or down-regulation by neighbor signals ($P < 0.044$, $q < 0.050$), excluding core genes, were classified in eight clusters using MultiExperiment Viewer (http://www.tm4.org) (Table S2).

*Binding target genes of transcription factors.* Percentage of binding target genes among core and specific gene was calculated using publicly available data for ELONGATED HYPOCOTYL 5 (HY5) (36), PIF3 (37), PIF4 (38), PIF5 (19), AUXIN RESPONSE FACTOR 6 (ARF6) and BRASSINAZOLE RESISTANT 1 (BZR1) (39). The statistical significance was tested using Fisher's exact test.

*Field microarray experiment.* In a previous work, we had reported the transcriptome of seedlings of the wild-type Columbia grown in the field under conditions of uninterrupted shade, shade daily interrupted by an afternoon sunfleck (starting 8 h after the beginning of the photoperiod of 10 h, and ending immediately prior to the beginning of the night) or uninterrupted sunlight (21). Here we add a fourth condition, corresponding to seedlings grown under sunlight interrupted daily by an afternoon shade event (starting 8 h after the beginning of the photoperiod of 10 h and ending immediately prior to the beginning of the night). These data correspond to samples obtained and processed simultaneously with the other three but not reported before. In all cases, two biological replicates were harvested in liquid nitrogen after 9 h of the beginning of the photoperiod of the third day of treatment. Other details were as described (21). We used factorial ANOVA with afternoon sunlight or shade and rest of the day sunlight or shade as main factors and selected the genes showing significant treatment effects ($P < 0.050$, $q < 0.050$) (30). We divided these genes in two groups, one corresponding to the core set and the rest corresponding to the context-specific genes. These groups were in turn assigned to two and four clusters using MultiExperiment Viewer (http://www.tm4.org) (Table S3).

## RESULTS

### A core set of genes that respond to neighbor signals in different contexts

We analyzed the normalized expression levels observed for each gene in the experiments described in Table 1 by ANOVA with light condition (control *versus* neighbor signal) and experiment as main factors. We first identified 3125 genes that showed significant effects of the light condition ($q < 0.050$). In a second step, we eliminated from this list any gene that at least in one of the experimental conditions failed to follow the average trend (either promotion or inhibition of expression by neighbor signals). This procedure defined a small set of 98 genes with enhanced expression in response to neighbor signals and 112

genes with reduced expression in response to neighbor signals independently of the context, which were defined as core neighbor signal genes (Fig. 1, Table S1).

Core genes up-regulated by neighbor signals include *LONG HYPOCOTYL IN FAR-RED* (*HFR1,* 11.3 ± 4.5, average fold change ±SE), *INDOLEACETIC ACID-INDUCED PROTEIN 29 (IAA29,* 5.9 ± 1.8) and *XYLOGLUCAN ENDOTRANSGLYCOSYLASE 7* (*XTR7,* 4.0 ± 1.1), which are binding targets of PIFs often used as markers of the shade-avoidance response (19). It also includes three photo-sensory receptors, *PHYA* (1.5 ± 0.2), *PHYB* (1.8 ± 0.3) and *PHOTOTROPIN 1* (*POHT1*, 1.3 ± 0.1) (40), and *PHYTOCHROME KINASE SUBSTRATE 2* (*PKS2,* 1.4 ± 0.1) (41).

One of the distinctive features of this group was the presence of a relatively large proportion of genes involved in hormone signaling. ABA signaling genes include *ABSCISIC ACID RESPONSIVE ELEMENTS-BINDING FACTOR 3 (ABF3*), *ABI FIVE BINDING PROTEIN 1* (*AFP1*) and 3 (*AFP3)*, and *G-BOX BINDING FACTOR 3 (GBF3*) (42–44). Auxin signaling genes include *INDOLEACETIC ACID-INDUCED PROTEIN 16* (*IAA16) and 29* (*IAA29)*, *SMALL AUXIN UP-REGULATED* proteins *SAUR7*, *SAUR23* and *SAUR66* and the auxin transporter *AUXIN RESISTANT 1 (AUX1)* (45–48). Brassinosteroid signaling genes include *BES1-INTERACTING MYC-LIKE PROTEIN 2 (BIM2*), *BES1/BZR1 HOMOLOG 4 (BEH4), ILI1 BINDING BHLH 1* (*IBH1*), *BRASSINOSTEROID-SIGNALING KINASE 6* (*BSK6)* and *BIN2-LIKE 2 (BIL2)*(49–52). Ethylene signaling genes include *EIN3-BINDING F BOX PROTEIN 2* (*EBF2*), *ERF TRANSCRIPTION FACTOR 9 (ERF9)* and *PAR1-RESPONSIVE 3* (*P1R3*) (53–55). Jasmonic acid signaling includes *JASMONATE-ASSOCIATED MYC2 LIKE 2* (*JAM2*) (56). Gibberellin-related genes include *GIBBERELLIN 2-OXIDASE 6 (GA2OX6)* (57). Noteworthy, 50% of these genes are related to transcriptional regulation and only one *(GA2OX6)* is a hormone metabolism gene. Hormone-related genes were also present among core genes down-regulated by neighbor signals, although with a much lower proportion than among up-regulated genes. For instance, ABA sensor protein *PYR1-LIKE 6 (PYL6*)(58); small auxin up-regulated protein *SAUR31*; ethylene response factor *TARGET OF EAT1 2 (TOE2*) (59); gibberellic acid signaling transcription factor *ARABIDOPSIS THALIANA HOMEOBOX PROTEIN 23 (ATHB23*) (60) and jasmonic acid biosynthesis gene *ALLENE OXIDE CYCLASE 1 (AOC1*) (61). Little is known about the function of several of these genes in shade avoidance.

Conversely, core down-regulated genes were significantly enriched in genes coding for chloroplast localized proteins (47.6% compared to 22.7% for genomic average, $P < 0.0001$), whereas among core up-regulated the proportion of genes coding for chloroplast localized proteins were significantly reduced (8.2%, $P < 0.001$). Genes involved in starch metabolism ($P <$ 1.40E-05, including the genes of the phosphoglucomutase STF1, the phosphoglucan water dikinase PWD and the disproportionating enzyme 2 DPE2) and heterocycle metabolism ($P <$ 3.58E-08, including the genes corresponding to the protochlorophyllide oxidoreductase PORC, the UbiA prenyltransferase family protein PDE325, the glutamate-1-semialdehyde 2,1-aminomutase GSA2, the adenine phosphoribosyl transferase 2 PHT1.1, the Uroporphyrinogen decarboxylase HEME2, the Flavin containing amine oxidoreductase family protein PPOX, non-photochemical quenching 1 NPQ1 and the tryptophan synthase beta type 2 TSBtype2) were enriched within the core down-regulated by neighbor light signal.

### Context-specific genes

For comparative purposes, we defined two set of context-specific genes. The first set corresponds to the frequent responders, which include all the genes that showed significant effects of neighbor signals and were excluded from the core set due to their response opposite to the general pattern in at least one condition. According to their average response, the frequent responders, context-specific genes were grouped in 1326 and 1589 genes predominantly up- or down-regulated by neighbor signals, respectively (Table S1). Frequently up-regulated genes are significantly enriched in genes involved in response to UV-B ($P <$ 2.25E-03), response to water deprivation ($P <$ 2.77E-10), disaccharide biosynthesis ($P <$ 1.35E-04), gravitropism ($P <$ 1.25E-03), disaccharide metabolism ($P <$ 1.19E-04), amino acid catabolism ($P <$ 6.95E-04), gibberellic acid-mediated signaling ($P <$ 3.66E-04) and auxin metabolism ($P <$ 2.94E-05). Frequently down-regulated genes are enriched in starch metabolism ($P <$ 1.46E-06), chloroplast organization and biogenesis ($P <$ 3.01E-07), pigment biosynthesis ($P <$ 2.75E-09) and amino acid biosynthesis ($P <$ 2.65E-14).

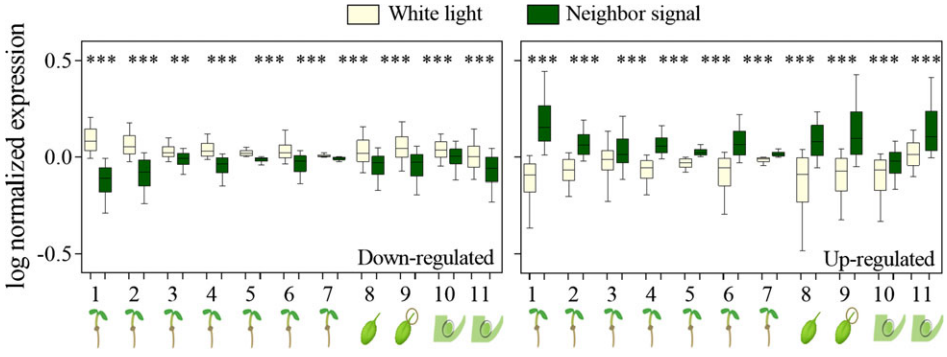


**Figure 1.** A core set of genes show consistent promotion or inhibition of expression by neighbor signals in different contexts. Box-plots show median, 1–3 interquartile range and 95% confidence interval of normalized expression values of 98 up-regulated and 112 down-regulated genes. The numbers in abscissas indicate the experimental condition described in Table 1, and the drawing refers to the organs involved in each case. The significance of Student's *t*-test is indicated. ***$P < 0.001$.

The second set of context-specific genes corresponds to the occasional responders; a group of 1667 genes that showed significant interaction between neighbor signal and experiments and no main effect of neighbor signal (Table S1). This group was significantly enriched biological functions like red or far-red light signaling pathway genes ($P < 4.13E-04$), jasmonic acid-mediated signaling pathway genes ($P < 9.04E-04$), flavonoid metabolism genes ($P < 9.13E-05$) and response to water deprivation genes ($P < 6.33E-09$). However, none of these genes changed consistently. Their expression was promoted under certain conditions and repressed or unaffected in others (Figure S1). The patterns do not clearly reflect the different organs used in transcriptome studies, indicating that they can show interaction with the specific growth conditions of the experiment.

### Transcription factor genes are over-represented within the core set up-regulated by neighbor signals

Core up-regulated genes were significantly enriched in transcription factors (18.4%) compared to the frequent responders subgroup of context-specific genes (11.4%, $P < 0.01$) and the genome (9.0%, $P < 0.01$). These include transcriptional regulators of hormone signaling (ABA, auxin, brassinosteroid, ethylene and jasmonic acid, see above), phytochrome signaling, cell cycle, cell growth and development. Transcription factors were close to the genomic proportion among down-regulated core genes (7.1%). Within the context-specific genes, those frequently down-regulated included less transcription factors than expected by chance, based on genomic data ($P < 0.05$, 11.4% and 4.0% of the genes among those frequently up or down-regulated by neighbor signals, and 7.2% among the genes occasionally regulated by neighbor).

### Hyper-represented transcription factor binding sites in core genes

To investigate whether core genes are subjected to a common transcriptional regulation, we analyzed the proportion of these genes that are binding targets of key transcription factors involved in light signaling and growth control, such as HY5 (36), PIF3 (37), PIF4 (62), PIF5 (19), ARF6 and BZR1 (39).

Both core- and frequent-responder genes up-regulated by neighbor signals showed an important enrichment in the number of direct targets of each one of these transcription factors, compared with the genomic average. Occasional responders showed numbers much closer to the genomic average. Noteworthy, in core genes the enrichment was significantly higher than in frequent responder genes ($P < 0.0001$) (Fig. 2). Remarkably more than 80% of up-regulated core genes are binding targets of PIF4, more than 65% are binding targets of ARF6 and BZR1 and more than 45% are binding targets of the BZR1, ARF6 and PIF4 tripartite module of interacting transcription factors (BAP module (39)). HY5 and PIFs form a dynamic activation-suppression transcriptional module by directly targeting a common promoter cis-element (63). Core genes up-regulated by neighbor signals were significantly enriched in shared HY5-PIF binding sites ($P < 0.05$). Most (91%) of the genes bound by HY5 are also bound by PIFs. Context-specific and core genes down-regulated by neighbor signals showed binding proportions close to the genome values (Fig. 2).

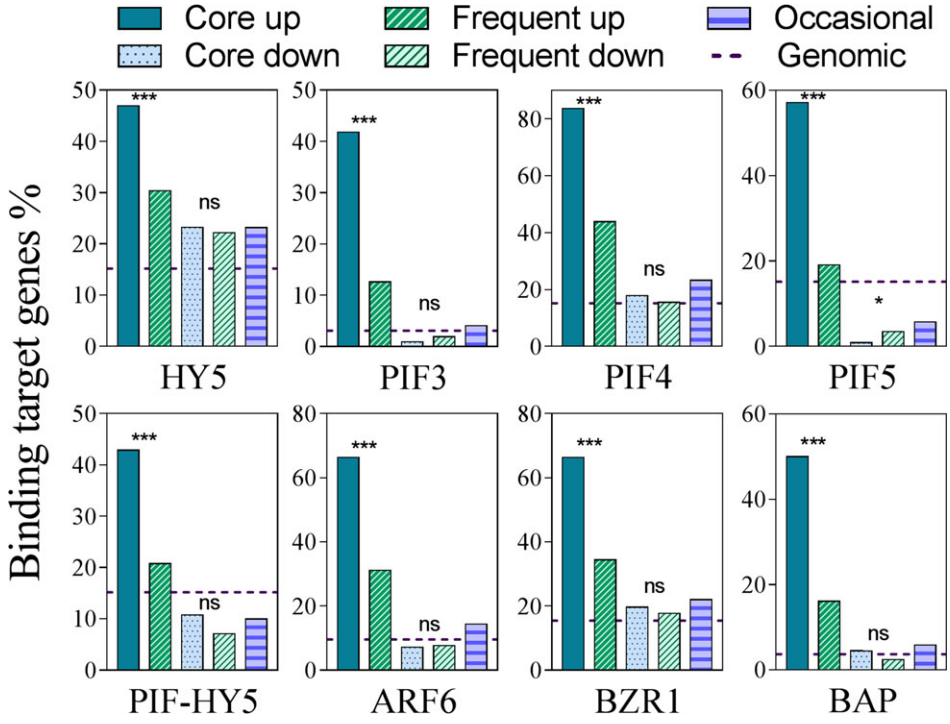


**Figure 2.** Proportion of binding target genes of HY5, PIF3, PIF4, PIF5, PIF3, 4 or 5 and HY5, ARF6, BZR1 or the BAP module within the core set of genes either promoted or inhibited by neighbor signals, the frequent responders either promoted or inhibited by neighbor signals, the occasional responders and the genome. The significance of Fisher's exact tests between core and frequent up-regulated genes and between core and frequent down-regulated genes is indicated. ***$P < 0.001$, *$P < 0.05$, ns, not significant.

### Genes with expression impaired in the *pif1 pif3 pif4 pif5* mutant

The proportion of genes with expression affected in the *pif1 pif3 pif4 pif5* mutant (24) shows the same general pattern observed for the binding of PIFs, that is high within the core set with positive response to neighbor signals and relatively lower in the rest (Figure S2). The proportions of impact on expression are lower than those observed for the binding by PIFs of these genes (compare Fig. 2 and Figure S2) and this might reflect either that not all the binding targets are always affected by PIFs (i.e. they are not true direct targets as defined by Pfeiffer *et al.* (64)) or differences in the experimental precision involved in the definition of binding and impact on expression. Previous reports have indicated that direct targets of multiple PIFs are enriched in regulation of transcription and plant hormone-associated functions as described here for the core up-regulated gene set (64).

The core presented here should not be confounded with that defined earlier (24). Leivar *et al.* (24) identified 103 genes rapidly (1 h) induced by low red/far-red in a PIF-dependent manner and subdivided this group into two subsets, one enriched in transcription factor genes and promoters containing G-box motifs and the other lacking G-box motifs and enriched for auxin-responsive loci. Of these subsets, only 14 and 6 genes, respectively, are present in our core with positive response to neighbors. They also identified 20 genes rapidly repressed by low red/far-red in a PIF-dependent manner, none of which is present in our core set with negative response to neighbors. Some of the genes selected by Leivar *et al.* (24) did not respond in other developmental contexts and are not included here. Other genes did not reach the cutoff criteria in Leivar *et al.* (24) and are included here thanks to the enhanced statistical power observed when a gene shows consistent responses across a large number of experiments.

### Neighbor signals modify hormone-responsive genes in a context-dependent manner

As core genes up-regulated by neighbor signals are enriched in binding targets of hormone-related transcription factors ARF6 and BZR1 and show a large proportion of hormone signaling genes, we investigated whether neighbor signals have general effects on the status of hormone signaling. For this purpose, we studied the expression of subsets of genes previously defined as responsive to auxins, gibberellic acid, brassinosteroids, abscisic acid, jasmonic acid or cytokinins (32). Neighbor signals affected the expression of each one of these hormone-marker groups (Fig. 3); however, the impact was context-specific as none of these marker groups was affected in all the experimental conditions involved in the current analysis.

The general trends were as follows: for auxin, ethylene and gibberellins, promoted and inhibited genes tended to be promoted and inhibited by neighbor signals, respectively, with one case in the opposite direction for gibberellins. Conversely, for cytokinin and methyl jasmonate, promoted and inhibited genes tended to be inhibited and promoted by neighbor signals, respectively, with one case in the opposite direction for methyl jasmonate. For abscisic acid and brassinosteroids, both promoted and inhibited genes tended to be promoted by shade. GA up-regulated genes where induced by natural shade and low red/far-red ratios in seedlings, whereas its expression was reduced by neighbor signals in leaf blade. GA down-regulated gene expression tended to be decreased by neighboring signals.

### Shared and differential transcriptome responses to neighbor signals, full darkness and night

To further characterize the core set of genes, we investigated their pattern of response to other conditions where the light input is affected, such as the transition between full darkness and light during de-etiolation and day/night cycles. Core genes up-regulated by neighbor signals were also repressed by light during de-etiolation and induced during the night (Fig. 4), indicating that this group is particularly sensitive to light conditions. Core genes down-regulated by neighbor signals showed the opposite pattern (although the response to long days and short nights was weak). For comparative purposes, we defined a set of genes that respond consistently to shade at the hypocotyl growth stage (17,21,24) and are not part of the core set (Table S2). This group of genes that respond to neighbor signals in a context-specific manner contained members that responded to de-etiolation and day–night conditions as core genes did (Fig. 4). The different clusters show diverse kinetics of response to day/night cycles including either progressive effects (clusters 1 and 5 and more moderate in cluster 2) or a more marked switch between day and night (clusters 4 and 8). Leivar *et al.* (24,33) defined a set of 30 genes displaying rapid (within 1 h) repression in response to continuous red light during de-etiolation and strong, rapid (within 1 h) induction in response to supplementary far-red light in de-etiolated seedlings. Twenty-eight of these 30 genes showing reciprocal responsiveness are included among core shade up-regulated (5 genes) and frequently shade up-regulated genes (23 genes, clusters 1, 3 and 4).

Interestingly, clusters 3 and 7 remained unaffected by the photoperiod (Fig. 4). This group of 1262 genes is specifically affected by neighbor signals, discriminating against the major changes in light input that occur during day/night cycles. Noteworthy, thylakoid membrane genes ($P$ = 2.75E-21) and structural constituent of ribosome ($P$ = 9.75E-29) are highly overrepresented in cluster 7. Finally, in cluster 6, expression is reduced by shade but enhanced by the night.

Several transcription factor binding sites are overrepresented within the genes that respond in the context of these hypocotyl stage experiments ((31), See Table S4). Some of them could correspond to the transcription factors present in the core set of genes.

### The response to dynamic neighbor signal conditions

To investigate the dynamics of the core set of genes under fluctuating neighbor signals, we analyzed the transcriptome of seedlings grown in the field (day/night cycles) under conditions of uninterrupted sunlight, sunlight interrupted daily by an afternoon neighbor shade event, uninterrupted neighbor shade and neighbor shade daily interrupted by an afternoon sunfleck (Fig. 5). In the seedlings exposed to dynamic signals, the expression of the core set of genes responded to the transition without reaching the levels observed in seedlings grown under stable sunlight or shade photoperiods (Fig. 5a). In other words, in these genes the expression levels represent a balance between current and previous conditions in the day. For comparative purposes, we defined the context-specific set of genes of this experiment as the genes

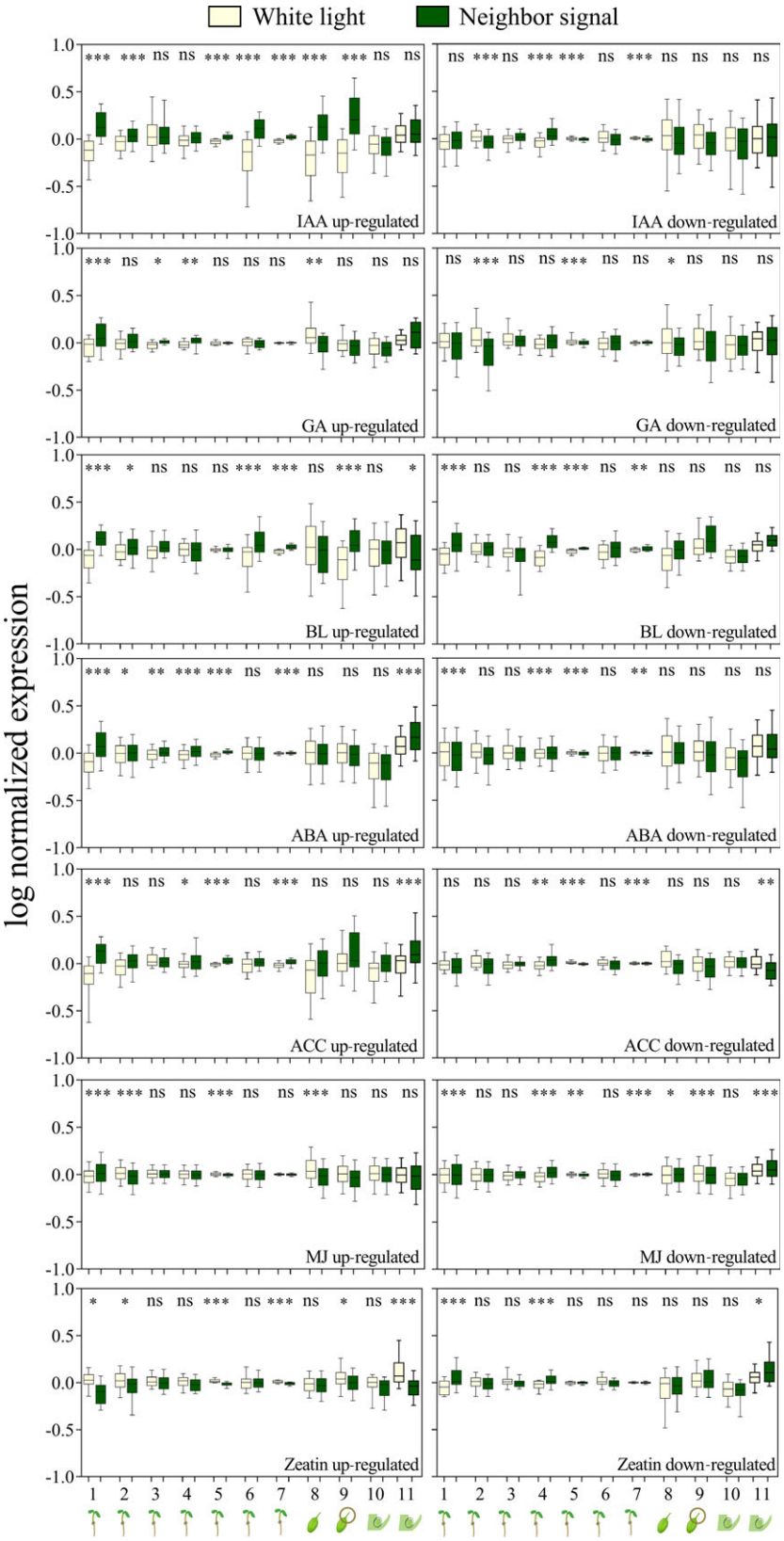


**Figure 3.** Expression of hormone response marker genes as affected by neighbor signals. Genes with expression promoted or inhibited by each hormone are indicated separately. Box-plots show median, 1–3 interquartile range and 95% confidence interval of normalized expression values. IAA, auxin, GA, gibberellic acid, BL, brassinosteroids, ABA, abscisic acid, ACC, ethylene, MJ, methyl jasmonate. The numbers in abscissas indicate the experimental condition indicated in Table 1, and the drawing refers to the organs involved in each case. The significance of Student's *t*-test is indicated. ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$, ns, not significant.

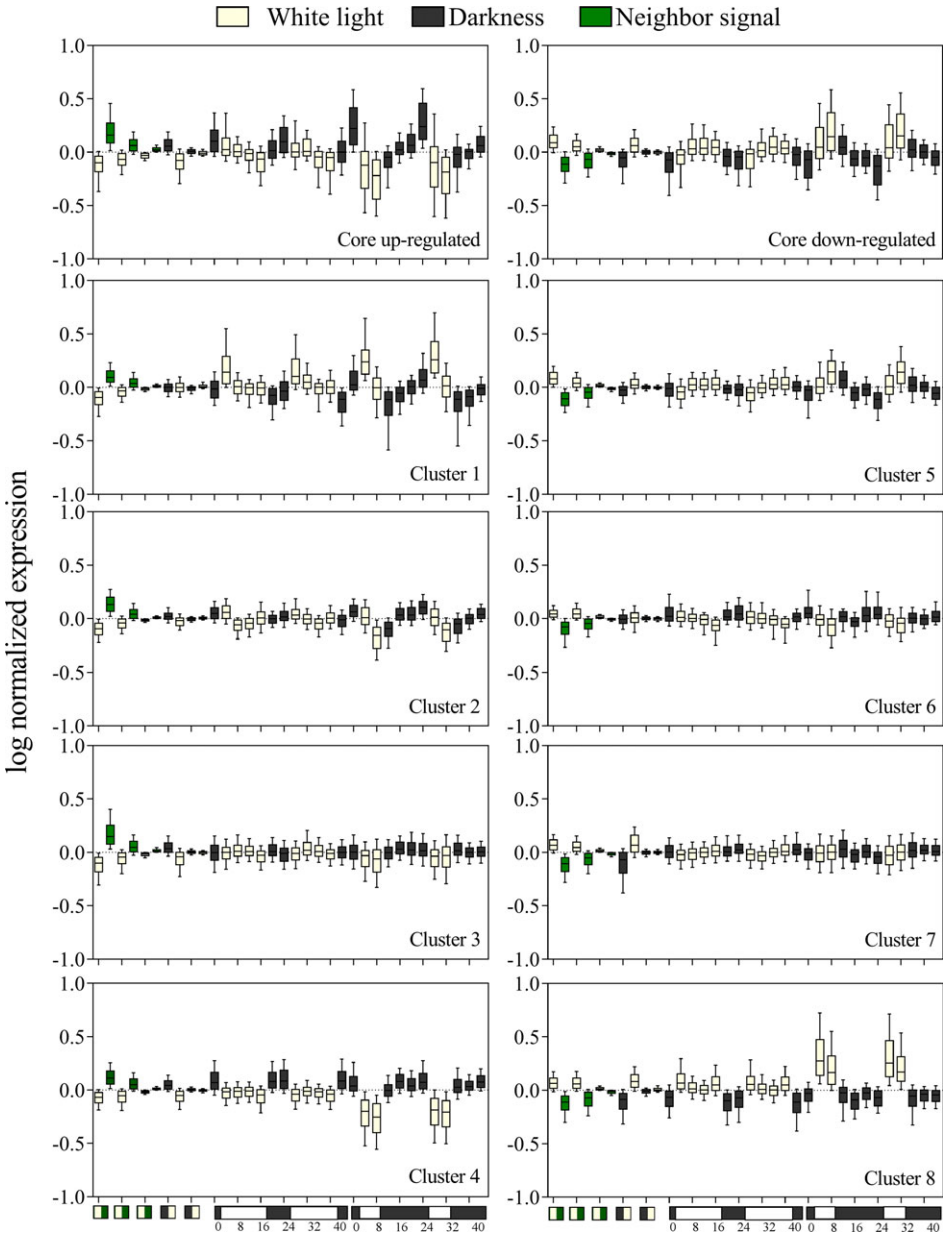


**Figure 4.** Dynamics of core- and context-specific genes in response to neighbor signals and dark/light transitions during de-etiolation and day–night cycles. Logarithm of normalized expression of core genes up- and down-regulated by neighbor signals and context-specific responder genes identified in experiments 1, 2 and 5 (Table 1) (17,21,24). The first six plots correspond to these three experiments, the following four correspond to two de-etiolation experiments (33,34), and the final 24 correspond to samples taken every 4 h during two long days and two short days (35). Box-plots show median, 1–3 interquartile range and 95% confidence interval of normalized expression values.

showing significant treatment effects ($P < 0.050$, $q < 0.050$) but not belonging to the core group. Clusters 1 and 2 of these context-specific genes showed patterns of response very similar to those of the core (Fig. 5a). Cluster 3 includes genes that enhance their expression only in response to interruptions of neighbor shade. Cluster 4 includes genes with expression affected by the current conditions, largely independent of their previous exposure to sunlight or neighbor shade during the early part of the day (Fig. 5).

Within the context-specific genes, the proportion of binding targets of HY5 (36), PIF3 (37), PIF4 (62), PIF5 (19), ARF6 and BZR1 (39) varied strongly among clusters and differentially for each transcription factor (Fig. 5b). This suggests that the combinatory control by the different transcription factors could be important to set the specific dynamics of gene expression response to fluctuating shade conditions.

## DISCUSSION

Following the meta-analysis of the expression data published by several laboratories using a common platform (17,19,21–27), we have identified a small set of genes, the core neighbor response genes, which respond consistently to neighbor signals. This finding is to some extent surprising because the samples were based

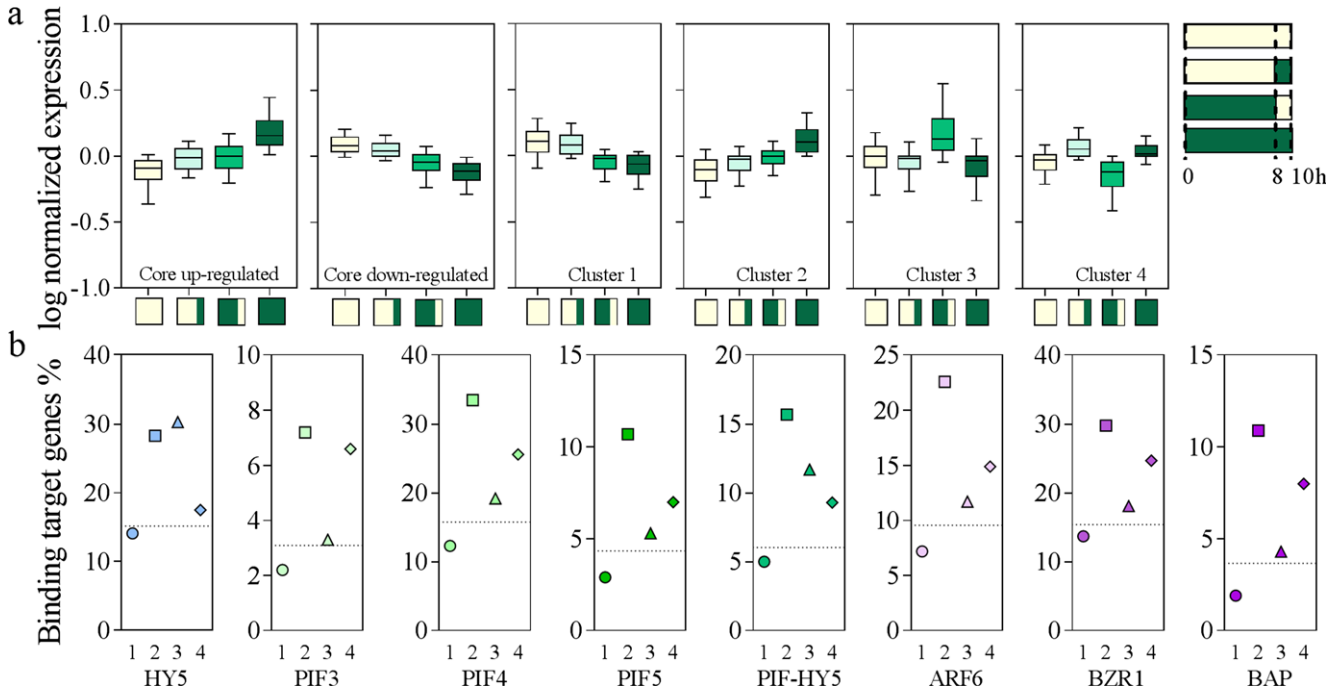


**Figure 5.** Dynamics of core- and context-specific genes in response fluctuating conditions of natural shade in the field. The seedlings were grown under uninterrupted sunlight, sunlight interrupted daily by an afternoon neighbor shade event, uninterrupted neighbor shade and neighbor shade daily interrupted by an afternoon sunfleck as represented in the diagram. (a) Logarithm of normalized expression of core genes up- and down-regulated by neighbor signals and context-specific responder genes identified in the field experiment and classified in four clusters. Box-plots show median, 1–3 interquartile range and 95% confidence interval of normalized expression values. (b) Proportion of binding target genes of HY5, PIF3, PIF4, PIF5, PIF3, 4 or 5 and HY5, ARF6, BZR1 or the BAP module within the four clusters shown in (a) (clusters indicated in abscissas).

on different organs, which even show contrasting growth responses to neighbor signals (Table 1). In addition, the experiments involve different approaches to simulate the light signals caused by the presence of neighboring vegetation, and experiments going from white light to neighbor signal conditions and vice versa. This core set of genes is relatively small (98 promoted and 112 repressed by neighbor signals) when compared to the context-specific genes, including those similarly affected in most but not all the experiments (1326 promoted and 1589 repressed by neighbor signals) and those that responded occasionally (1667 genes). The core set of genes provides an extensively corroborated list of markers of the response to neighbor light signals. It shows little overlap with groups defined earlier using different criteria (24).

The core set of genes shows reversible responses to neighbor signals because they were selected from experiments describing a shift in conditions in one direction or the other (Table 1). The actual kinetics of the response in each direction is in average rather symmetric (Fig. 5). The way the expression of core genes follows light conditions is not limited to the presence or absence of neighbor signals because they also respond to the dark or light conditions during de-etiolation and during day–night cycles (Fig. 4).

Within the core, there is a marked asymmetry between up- and down-regulated genes. The binding targets of PIFs are very highly overrepresented within the core set of genes with a positive response to neighbor signals (Fig. 2). This is true not only when compared to the rest of the genome or to the occasional responders but also when compared to the genes that respond in most although not all the contexts. The binding targets of other members of the BAP module, which integrates hormonal and external cue pathways in the regulation of genome expression and growth (39), were also overrepresented among the core set of genes. Noteworthy, the binding targets of HY5 were also over-represented among the core genes up-regulated by neighbor signals, suggesting that HY5 might balance gene expression against the action of PIFs as reported for pigment biosynthesis (63). Conversely, the binding targets of PIFs, other members of the BAP module and HY5 are not over-represented among the core genes that reduce their expression in response to neighbor signals (Fig. 2).

Within the core, up- and down-regulated genes also have different function. Among the core genes with positive response to neighbor signals, we can find three photoreceptors (*PHYA*, *PHYB* and *PHOT1*), *HFR1* (18,23) and *PKS2* (41), suggesting that one of the general functions of this subset is to control the sensitivity to light signals. Starch metabolism and heterocycle metabolism genes are overrepresented within the core genes with negative response, suggesting that these functions are down-regulated by signals of the presence of neighbors, which may actually reduce the photosynthetic input. Furthermore, the proportion of transcription factors found within the core genes with positive responses to neighbors is twice that observed in the genome, but this is not the case among negative responders. Finally, several core genes with positive response to neighbors are related to hormone signaling or perception, suggesting that modification by light cues of the signaling steps downstream the hormone itself might be a mechanism more frequent than acknowledged so far.

Key functions in the response to shade are out of the core set of genes. The phyB-PIF-YUCCA module that drives the

promotion of auxin synthesis in response to neighbor signals is the best established in the control of shade avoidance (14,19). Despite the strong link between PIFs and the core set of genes with positive response to neighbor signals, they only show one gene involved in the metabolism of hormones and it is not a *YUCCA* or auxin-related gene. This is consistent with the reported occurrence of shade-avoidance responses in the absence of obvious changes in auxin levels (25,65,66). We have analyzed markers of the response to different hormones (auxin, gibberelling, brassinosteroids, abscisic acid, ethylene, cytokinins) (32). Each one of these groups showed effects under certain conditions, but none of them showed a response in all cases. Therefore, although neighbor signals tend to modify the hormone signaling status, they do so in a context-dependent manner.

Other functions out of the core include response to UV-B, response to water deprivation, disaccharide biosynthesis, amino acid catabolism and auxin metabolism within frequently up-regulated genes; and starch metabolism, chloroplast organization and biogenesis, pigment biosynthesis and amino acid biosynthesis within frequently down-regulated genes.

The specificity of response to neighbor signals is also out of the core set. Among the genes able to respond to neighbor signals at the hypocotyl stage (i.e. in a context-dependent manner), there are groups that respond only to neighbor signals and are unaffected during de-etiolation or during day–night cycles (Fig. 4). This is interesting because plants are much more sensitive to neighbor signals during the day than when artificially provided during the night (8), suggesting that this feature would not result simply from the dynamics of the core set of genes themselves but imposed by the specific transcriptional context.

In conclusion, we propose that the structure of the transcriptional circuit of response to neighbor signals involves four major tiers:

1 The core set of genes up-regulated by neighbor signals. The reduced activity of phytochromes (67) and cryptochromes (15) in response to neighbor signals enhances the activity of PIFs. Therefore, the entry point of the photo-sensory receptor signal to the transcriptional circuit would be formed by PIFs and their entourage of binding targets. The main functions of this set would be the control of light sensitivity and downstream transmission of the transcriptional wave favored by the large proportion of transcription factors. This task would involve transcription factors known to act in hormone signaling but without necessarily mimicking the hormone response.
2 The core set of genes down-regulated by neighbor signals. These genes involve functions in starch and heterocycle metabolism. The connection to the photo-sensory receptor signals is not clear. These genes might be controlled by the transcription factors present within the core set of up-regulated genes and/or by unidentified players downstream phytochrome and cryptochrome. For instance, *SQUAMOSA PROMOTER-BINDING PROTEIN LIKE 13 (SPL13)* is present in the core with positive response and SQUAMOSA promoter-binding protein (SBP) box is overrepresented among the core down-regulated genes (3 E-5 (31)).
3 Beyond the core, we can find the genes that respond in a context-dependent manner. Some of the most important functions during the response to shade are related to these genes. Those with positive response could be controlled by the photo-sensory receptors directly via PIFs (Fig. 2) or indirectly via the transcription factors present in the core of up-regulated genes (Table S4).
4 Finally, we can find a set of genes with volatile performance, which respond occasionally and in opposite directions to neighbor signals depending on the context.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

**Table S1.** List of core, frequent responder and occasional responder genes obtained by the meta-analysis of the transcriptome as affected by light signals of neighbors.

**Table S2.** List of genes included in the clusters described in Figure 4.

**Table S3.** Normalized expression obtained in field microarray experiment comparing the wild type grown under uninterrupted shade, shade daily interrupted by an afternoon sunfleck, uninterrupted sunlight and sunlight daily interrupted by an afternoon shade event.

**Table S4.** Motifs overrepresented among the promoters of the genes present in the clusters of context-specific genes shown in Figure 4.

**Figure S1.** Genes showing occasional responses to neighbor signals.

**Figure S2.** Proportion of genes with expression enhanced or reduced in the *pif1 pif3 pif4 pif5* mutant (24) within the core set of genes either promoted or inhibited by neighbor signals, the frequent responders either promoted or inhibited by neighbor signals, the occasional responders and the genome.

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