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Original Article

Mutants of phospholipase A (pPLA-I) have a red light and auxin phenotype

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ABSTRACT

***pPLA-I* is the evolutionarily oldest patatin-related phospholipase A (pPLA) in plants, which have previously been implicated to function in auxin and defence signalling. Molecular and physiological analysis of two allelic null mutants for *pPLA-I* [*ppla-I-1* in Wassilewskija (Ws) and *ppla-I-3* in Columbia (Col)] revealed *pPLA-I* functions in auxin and light signalling. The enzyme is localized in the cytosol and to membranes. After auxin application expression of early auxin-induced genes is significantly slower compared with wild type and both alleles show a slower gravitropic response of hypocotyls, indicating compromised auxin signalling. Additionally, phytochrome-modulated responses like abrogation of gravitropism, enhancement of phototropism and growth in far red-enriched light are decreased in both alleles. While early flowering, root coils and delayed phototropism are only observed in the Ws mutant devoid of phyD, the light-related phenotypes observed in both alleles point to an involvement of pPLA-I in phytochrome signalling.**

Key-words: *Arabidopsis*; gravitropism; patatin-related phospholipase A; phototropism; phytochrome; root coiling; shade avoidance.

INTRODUCTION

Phospholipase A (PLA) hydrolyses phospholipids either at the hydroxyl group of the C_1 (PLA_1) or of the C_2 atom (PLA_2) and liberates free fatty acids and lysophospholipids as products. Several structurally different enzymes can show this activity in plants (Scherer 2010; Scherer *et al*. 2010). These enzymes are the small (14 kDa) secreted PLA_2 ($sPLA_2$) (Ståhl *et al*. 1999), also found in fungi and animals, and the larger soluble patatin-related PLA (pPLA), which encompass the homologous soluble calcium-independent PLA_2 ($iPLA_2$) in animals (Six & Dennis 2000; Balsinde & Balboa 2005) and patatin-related PLA (pPLA) (Scherer *et al*. 2010). pPLAs hydrolyse fatty acids of both phospho- and galactolipids (Matos *et al*. 2001; Yang *et al*. 2007). The plant *pPLA* gene family is divided into three subfamilies (Holk *et al*. 2002; Scherer *et al*. 2010). The enzymes of the pPLA groups II and III with no additional domains have a molecular weight of around 50 ± 5 kDa. Proteins of subfamilies II and III do not have domains besides the enzymatic domain itself and can be distinguished by overall homology and their exon–intron structure. Group I in *Arabidopsis* comprises only one gene (*At-pPLA-I*) having an additional N-terminal leucin-rich repeat (LRR) domain with a G-protein-binding motif within this LRR domain, and a C-terminal domain with unknown function. Gene *pPLA-I* is the one gene most similar to the homologous so-called calcium-independent PLAs (iPLA) described for animals (Winstaed *et al*. 2000; Holk *et al*. 2002), and therefore, probably the evolutionarily oldest *pPLA* gene in plants.

Our laboratory investigated the function of auxin-activated and elicitor-activated patatin-related PLA (pPLA). Activation of pPLA by auxin is detectable after 2–5 min, depending on the method of measurement (Scherer & André 1989; Paul *et al*. 1998). pPLA blockers inhibit activity of purified recombinant patatin-related pPLA, auxin activation of pPLA activity *in vivo*, elongation growth (Scherer & Arnold 1997; Holk *et al*. 2002) and auxin-induced proton secretion (Yi *et al*. 1996). Moreover, auxin regulation of the *DR5* promoter and of several *IAA* genes is sensitive to pPLA inhibitors (Scherer *et al*. 2007). Three group II pPLAs can be phosphorylated at a serine in the C-terminus, and this phosphorylation enhances the pPLA-IIδ and pPLA-IIε activity *in vitro*. Moreover, *pPLA-IIε* knockout mutants have fewer lateral roots, and a *pPLA-IIγ* null mutant does not respond to phosphate deficiency (Rietz *et al*. 2010) by forming lateral roots and decreasing the main root length, both being typical responses to phosphate deficiency (Pérez-Torres *et al*. 2008). *pPLA-IIδ* null mutants have an auxin hypersensitive-like root phenotype (Li *et al*. 2011; Labusch *et al*. 2013). Several laboratories have demonstrated rapid activation of pPLAs in defence-related processes. Plant defence elicitors were shown to induce the production of free fatty acids and lysophospholipids within minutes in tomato leaves (Narváez-Vásquez *et al*. 1999), *Eschscholtzia*

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californica cells (Viehweger *et al.* 2002, 2006) and *Petroselinum crispum* cells (Scherer *et al.* 2000, 2002). Viehweger *et al.* (2006) were the first to show that elicitors induce a transient rise of lysophosphatidylcholine (LPC), which is followed by activation of a Na^+/H^+ exchange transporter, which thus acidifies the cytosol. LPC is also a second messenger in mycorrhiza formation (Drissner *et al.* 2007). Taken together, all previous works point to functions of plant pPLAs in auxin and defence signalling.

Because of the genetic resundance of 10 *pPLA* genes in the *Arabidopsis* genome, a functional analysis of individual genes is needed. The first knockout mutant plants of the *pPLA-I* gene of this gene family, *pplaI-1* and *pplaI-2*, were described by Yang *et al.* (2007). The T-DNA insertion mutants of *pPLA-I* investigated here, *pplaI-1* (in Ws) and *pplaI-3* (in Col), do not exhibit an obvious growth defect when grown in soil or on agar plates. However, we found the mutants to have a complex phenotype regulation of delayed early auxin-responsive genes, delayed gravitropism in darkness and decreased abrogation of gravitropism by far red (FR) in both alleles as well as and delayed phototropism in *ppla-I-1*. Coil formation was found in *ppla-I-1* and *phyB* but not in *ppla-I-3*. Interestingly, both mutants showed a hypersensitive growth response in FR-enriched light pointing to compromised and low output of phyB signalling.

RESULTS

Small differences in phenotypes of *ppla-I-1* and *ppla-I-3* are not explained by structural difference in gene or mRNAs

We isolated two T-DNA insertion mutants for the *pPLAI* gene, *pplaI-1* (Ws background) and *pplaI-1* (Col background). For *pPLA-I* an early annotation lists a sequence with 17 exons (NM_104867.3), whereas the newest annotation predicts 18 exons in the gene and two splicing sites different from the earlier version (NM_104867). During the course of our work, we noted some differences between the two mutant lines, which might have been due to sequence or splicing differences. This prompted us to sequence the genomic loci and cDNAs of both lines, which proved to be identical to the latest annotation (18 exons each; Fig. 1 a). Yang *et al.* (2007) described the sequence of a BAC clone CD4–16 encompassing the *pPLA-I* cDNA with exon4 missing, and used this clone for the expression of the pPLA-I protein. We found, however, that this clone lacked exon7, not exon4, and contained 19 single base errors when compared with the genomic sequence, apparently introduced by the reverse transcriptase used to create the clone. According to our sequence data of CD4–16, it can code for a protein of about one-third of the complete protein, only containing several amino acid changes in that fragment. After that, a stop codon would terminate this potential protein (Fig. 1b). The missing exon7 in CD4–16 could be due to a splicing error. We tested this by choosing primers for quantitative RT-PCR in the upstream 5′-terminal part of the RNA, for the exon7 alone, and for the downstream 3′-terminal part of the RNA. In both Ws and Col wild type (WT) the exon7 was present in mRNA at levels 25 times lower than the 5′-terminal part and about eight times less than the 3′-terminal part (Fig. 1c). From this we conclude that mis-splicing, resulting in the excision of exon7, occurs 25 times more often than formation of the complete mRNA productive for protein biosynthesis. The reason for not coding a full protein is that the potential translation of mRNA without exon7 is also terminated prematurely in a stop codon. We cannot exclude that the full-length mRNA transcribed from the *pPLA-I* gene was present in low abundance in the CD4–16 clone, but despite much effort, the missing exon7 was not found there and CD4–16 was not useful for protein expression. In conclusion, differences in *pPLA-I* gene or mRNA sequence, or in RNA splicing, are not a cause for the differences observed in the mutant lines *ppla-I-1* or *ppla-I-3*.

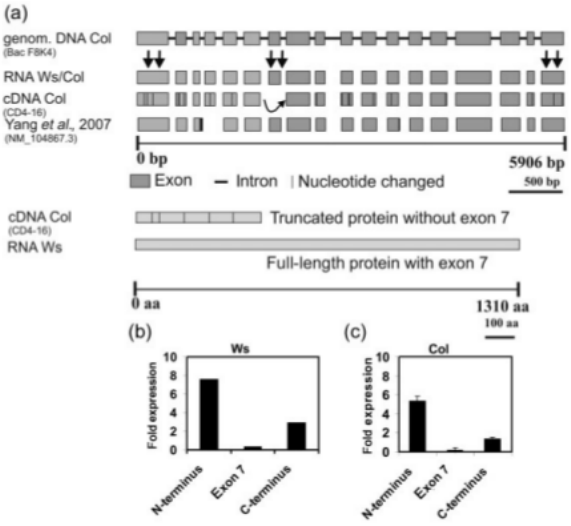


Figure 1. Genomic and exon–intron structure, mRNA sequences and splicing and transcription of *pPLA-I*. (a) Genomic sequence, known and postulated RNA sequences, and deduced protein sequences. A genomic BAC clone [BAC F8K4, source Col wild type (WT)] was used to isolate large fragments, which were sequenced and aligned by us. From both Ws and Col WT large fragments of cDNAs sequences were isolated and combined and gave the mRNA sequence by comparison with the genomic sequence. From a lambda cDNA library (CD4–16) made from Col WT the sequence was constructed from isolated fragments and a protein sequence deduced by us differing from the one described as NM_104486.3 (Yang *et al.* 2007). Our isolate from library CD4–16 was sequenced by us and the predicted protein sequence is given and below the protein sequence predicted by the last entrance in the The Arabidopsis Information Resource database. (b) Schematic presentation of potential protein sequences translated from the above mRNAs. (c,d) Relative amplicon frequencies as derived from the RNAs from Ws and from Col.

Expression pattern of pPLA-I

Both mutant lines are null mutants (Fig. 2a & b). We isolated Col plants transformed by a p_{AtPLAI}*:uidA* construct linking

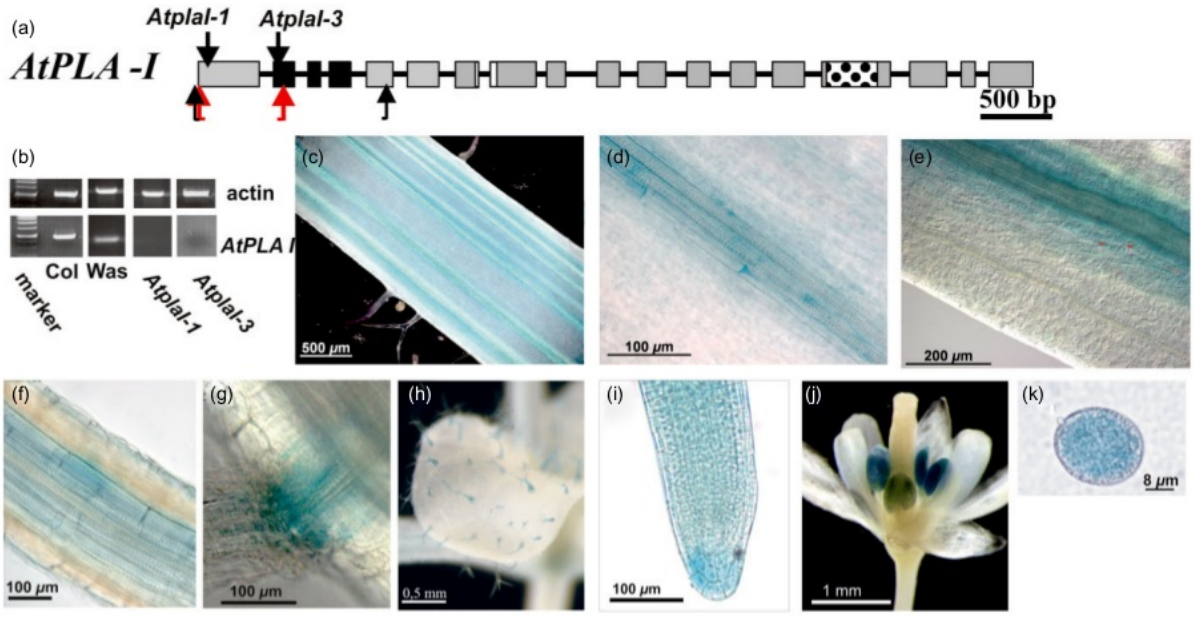
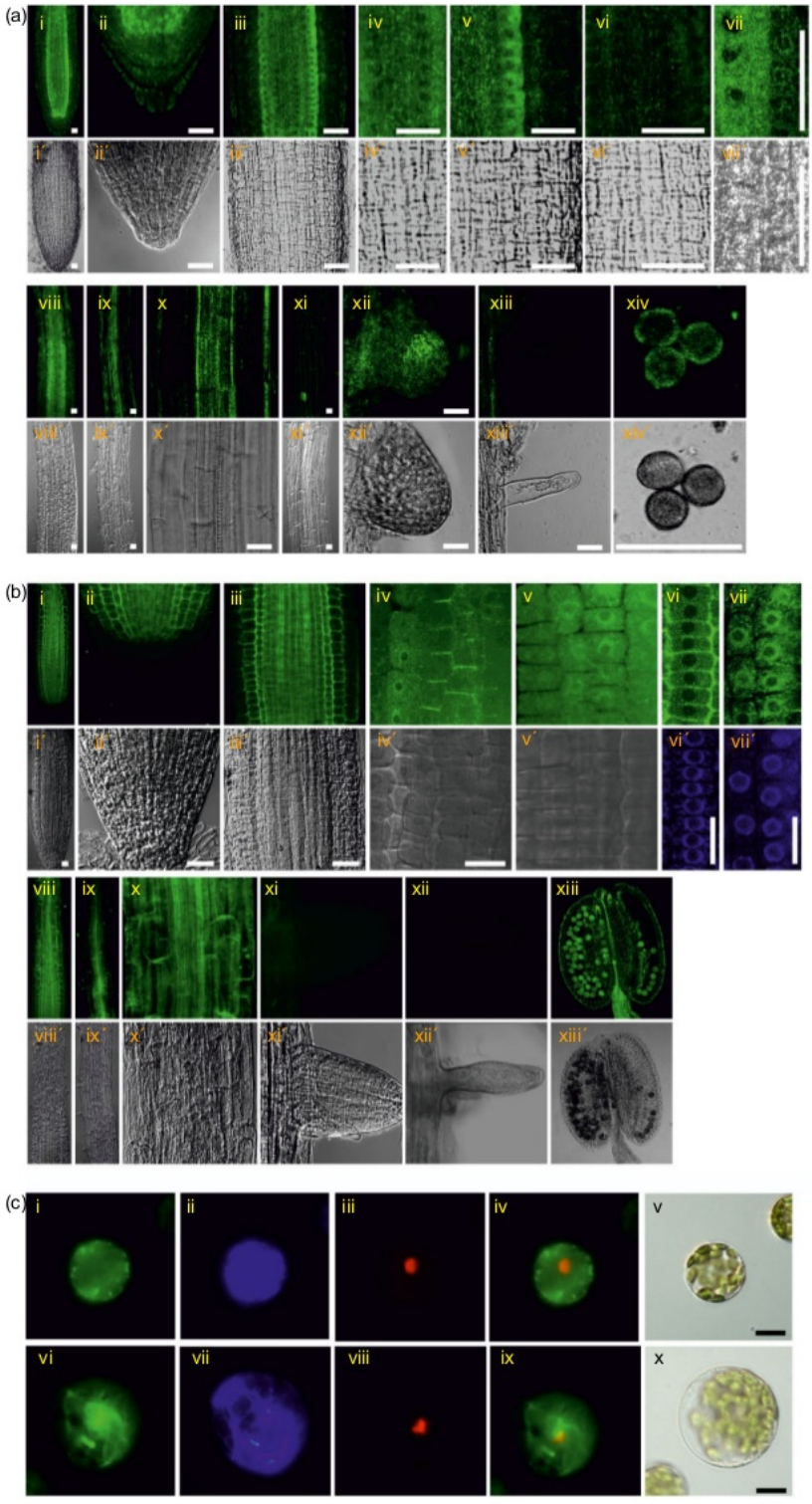


Figure 2. Expression of *pPLA-I*. (a) Exon–intron structure of the *pPLA-I* gene. Black boxes highlight the LRR structure; the small white boxes the catalytic center. Black arrows for insertions, red arrows for RT-PCR as shown in (b). (b) RT-PCR on 1-week-old seedlings of *pPLA-I* expression in Col, Ws, *pplaI-1* and *pplaI-3*. GUS expression overview from P_{PLAI}::*uidA* (c–k). (c) P_{PLAI}::*uidA* expression in the vascular of the inflorescence stem; (d) in a single the bundle of an inflorescence stem; (e) in a bundle of a petiole; (f) in the stele of the root; (g) in emerging lateral root; (h) in trichomes, (i) in the root tip; (j) localization of P_{PLAI}::*uidA* in the flower; and (k) in the pollen.

the *uidA* reporter gene to the promoter of the *AtPLA-I* gene. Prolonged (8 h) staining indicated p_{AtPLAI}-directed GUS activity in the vascular bundles of mature leaves and in pollen and trichomes only (Fig. 2e,h,i,j). With extended staining up to 48 h, the stem vascular bundles and the root stele were also stained (Fig. 2c,d,f,g), as was the columella, albeit weakly (Fig. 2i). At the site of lateral root emergence, we found stronger GUS activity in the stele of the newly formed lateral roots (Fig. 2g).

Tissue and subcellular localization was also investigated in plants expressing $_{PpPLA-I}$:*pPLA-I-GFP* in *ppla-I-1* (Fig. 3) and *ppla-I-3* background (Supporting Information Fig. S1) showing essentially the same tissue patterns. pPLA-I-GFP was expressed in the root tip and weakly in the columella (Fig. 3A, a & b), and there less in the rhizodermis and cortex, but strongly in the stele and endodermis (Fig. 3A, c–g). Expression decreased towards the root base (Fig. 3A, h–k). Young lateral root tips showed clear expression in the tip meristem (Fig. 3A, l). In root hairs, no expression was apparent (Fig. 3A, m), but strong in pollens (Fig. 3A, m). At higher magnification (Fig. 3A, g), a perinuclear localization, probably at the endoplasmic reticulum and nuclear membrane became visible. This could be observed clearly in endodermis cells, but is less intense in cortex cells exhibiting weaker expression. In addition, we found pPLA-I localization in the cytosol and near the plasma membrane. In the more mature parts of the root, in the transition from the meristematic part to the elongation zone and in the elongation zone, pPLA-I-GFP was found near the plasma membrane and not perinulear (Fig. 3A, j). Expression in the *ppla-I-3* background was nearly identical (Supporting Information Fig. S1).

Figure 3. Tissue and subcellular localization of pPLA-I. (a) pPLA-I-GFP expression in *ppla-I-1* background. Plants were grown in white light for 7 d. (i) root tip; (ii) quiescent centre with root cap; (iii) mitotic zone in root tip; (iv) stele lined by endodermis on both sides; (v) stele, endodermis, cortex and rhizodermis; (vi) rhizodermis flat section; (vii) row of endodermis and cortex cells (right); (viii) transition of elongation zone towards maturation zone; (ix) elongation zone; (x) elongation zone at higher magnification; (xi) mature zone; (xii) lateral root tip; (xiii) root hair; and (xiv) pollen. Bars: 20 µm. (i′–xiv′) light microscopy of otherwise identical settings. (b) Expression of pPLA-I determined by antibody against an N-terminal peptide in Ws seedlings. Plants were grown in white light for 7 d. (i) root tip; (ii) quiescent centre with root cap; (iii) mitotic zone in root tip; (iv) cross section (left to right): epidermis, cortical cell row, endodermis; (v) flat section of cortical cells; (vi) endodermis (compared with 4′,6-diamidin-2-phenylindol (DAPI) staining in vi′); (vii) cortical row (compared with DAPI in vii′); (viii) transition of elongation zone towards maturation zone; (ix) elongation zone; (x) elongation zone at higher magnification; (xi) lateral root tip; (xii) root hair; and (xii) pollen. Bars: 20 µm. (i′–xii′) light microscopy of otherwise identical settings. (c) Transient pPLA-I-GFP expression in *Arabidopsis* mesophyll protoplasts and coexpression of markers. (i, vi) pPLA-I-GFP; (ii, vii) ER-CFP marker; (iii, viii) NLS-RFP; (iv, ix) merger of pPLA-I-GFP; and ER-CFP (v, x) differential interference contrast light microscopy.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Localization was confirmed by immunocytochemistry with the anti-C-terminal antibody in the Ws WT (Fig. 3B). Results with the anti-N-terminus peptide-antibody were indistinguishable as were the results with the antibodies using Col WT plants (Supporting Information Fig. S2). Controls done with *ppla-I-1* and *ppla-I-3* plants showed no binding (Supporting Information Fig. S3). In the meristematic part of the root tip, all cell types expressed pPLA-I (Fig. 3B, a–g). The rhizodermal cells showed only weak expression. In the root cap, pPLA-I was not detected. Expression at the upper part of the meristematic zone decreased, and then, in the beginning of the elongation zone, was mainly found in the central cylinder (Fig. 3B, h–j). Localization within cells was found in the cytosol, near the plasma membranes and in a perinuclear ring-like distribution in some cell types, but especially in the subepidermal cortical cells (Fig. 3B, f & g). Comparison with 4′,6-diamidin-2-phenylindol (DAPI) staining verified the perinuclear staining (Fig. 3B, f′ & g′). Strong association with the plasma membrane was found in the endodermis (Fig. 3B, d & f).

In order to corroborate the subcellular localization of pPLA-I, we co-transfected p_{35S}*:: PLA-I:GFP* with either p_{35S}*::ER:CFP or* p_{35S}*::GOLGI:CFP* (Nelson *et al.* 2007) and p_{35S}*:: VirD2:mCHERRY* (Chakrabarty *et al.* 2007) into *Arabidopsis* (Col) mesophyll cell protoplasts. We also included pBIN-P19 (Silhavy *et al.* 2002) in some transformations to enhance expression of the transgenes. Fluorescence of PLA-I-GFP fusions was visible in the cytoplasm of transformed protoplasts, in some instances, fluorescent foci could be observed. In cases the Golgi and endoplasmatic reticulum marker protein – cyano-fluorescent protein fusion proteins – were co-expressed, very similar localization patterns of the marker proteins and the PLA-I-GFP fusion protein was apparent. No localization in the nucleus was observed. Tissue localization of GUS activity and by pPLA-I-GFP and immunocytochemistry using antibodies were in good agreement with each other. Expression in main root and lateral root caps and in trichomes was not found by immuno-detection, but because the whole mount labelling method requires cell walls, digestion properties in those cell walls could have retarded digestion.

ppla-I mutants have a dynamic auxin response phenotype

When seedlings were grown in white (W) light or darkness, no obvious differences between the respective WT and *ppla-I-1* and *ppla-I-3* mutant lines were observed. When tested on their response to auxin, no obvious differences in main root length or lateral root formation were found between mutants and respective WT. Therefore, the expression of early auxin-activated genes in both mutant lines and WT was tested to monitor a potential auxin response (Fig. 4). Rapidity of expression of the candidate genes regulation by exogenous auxin was chosen as a main criterion (Effendi *et al.* 2011), and the selected candidate genes fall into three groups: *IAA* genes, some of which are involved in the formation of lateral roots (Fig. 4a); *SAUR* genes (Fig. 4b); and *PIN2*, *PIN3*, and *GH3.5* (Fig. 4c). Thirty minutes after the application of 10 μM, 10 of 18 genes tested IAA in *ppla-I-1* (Ws), and 11 in *ppla-I-3* (Col) were induced significantly more weakly than in the corresponding WT.

In order to narrow down the time span during which pPLA-I could exert an influence on TIR1-directed transcription of early auxin genes, we investigated the expression of selected genes in the knockouts also after 10 min (Fig. 4d). In both alleles, delayed up-regulation of *IAA19*, *SAUR9* and *SAUR15* could be observed even in this short time window. *pPLA-I* expression itself is not influenced by auxin after 10 min (Fig. 4e), and only slightly after 30 min, excluding transcriptional regulation of *pPLA-I* as the cause of the diminished auxin-induction effect on early auxin-induced genes. When basal expression in untreated Col and Ws WT were compared, only *PIN2* was clearly differently expressed (25×) while others were only about twofold differently expressed (*IAA13, SAUR15, PIN3*; Fig. 4f). Together, despite the absence of an obvious phenotype, both mutant lines show a clear phenotype in delayed auxin-induced expression of key auxin induceable genes.

Phototropism and gravitropism are impeded and root coiling is increased in *ppla-I* mutants

When both mutant lines were illuminated with unilateral blue light after 3 d in D (dark), only the *pplaI-1* mutant showed a delayed phototropic response, but *pplaI-3* did not, when compared with the respective WT (Fig. 5a & b). After excluding sequence differences as potential cause for different phenotypes in the two alleles we speculated that the lack of phytochrome D (Aukerman *et al.* 1997) could be the cause for such dissimilarities. Phototropism can be promoted by switching off negative gravitropism in FR by phyA (Lariguet & Fankhauser 2004). Therefore we included *phyA* and *phyB* null mutants in the analysis. Indeed, the *phyA* mutant showed a reduced phototropic reaction, similar to *ppla-I-1*, whereas *phyB* reacted indistinguishable from WT (Fig. 5c & e).

In the dark hypocotyls of *pplaI-1* and *ppla-I-3*, the response to a gravitropic stimulus was considerably slower than the respective WT (Fig. 6a & b). After 24 h the average bending angle of the roots of dark-grown *ppla-I-3* seedlings was statistically indistinguishable from WT, while the one of *ppla-I-1* was slightly smaller than that observed of WT roots (Fig. 5c & d). Hypocotyl gravitropism is decreased by R and FR light (Robson & Smith 1996; Fankhauser & Casal 2004) and in *pif1pif4* double and *pif1pif3pif4pif5* quadruple mutants (Shin *et al.* 2009; Kim *et al.* 2011). The *pif* quadruple mutant hypocotyls are agravitropic in the dark indicating phytochromes as light receptors and their downstream transcription factors to be involved in gravitropism regulation. Therefore, we tested gravitropism in *phyA* and *phyB* seedlings under the same conditions as for *ppla-I* alleles. Only *phyB* hypocotyls showed a clearly reduced gravitropic response, whereas *phyA* roots and hypocotyls and *phyB* roots were indistinguishable from or very similar to WT in their responses (Fig. 6e–h).

Furthermore, we investigated the inhibition of hypocotyl gravitropism by FR light (Fankhauser & Casal 2004). In *phyB*

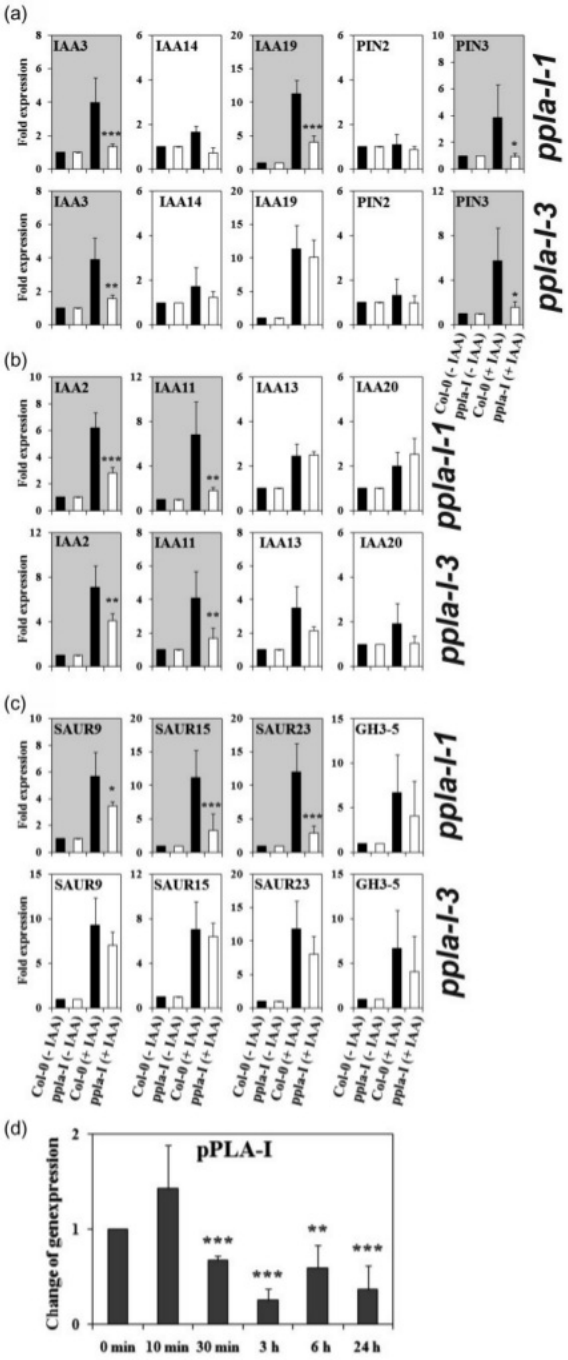

Figure 4. Regulation of early auxin-inducible genes in light-grown *ppla-I-1* and *ppla-I-3* knockout mutant lines and respective WT seedlings. The results are arranged into different groups of early auxin-inducible genes and other genes. (a) Regulation of selected *IAA* genes. (b) Transcription of *SAUR* genes (c) Expression levels of *PIN* genes and *GH3–5*. Black bars: auxin-treated 30 min; white: non-treated 0 min. (d) Responses after 10 min in selected genes. Grey background in panels highlight significant differences WT versus mutant lines. (e) Decrease of transcription of *pPLA-I* after extended treatment with 10 μM IAA (measured in WS background). (f) Comparison of WT Col (grey bars) and Ws (black bars) od all tested genes in non-treated samples. *PIN2* was about 30-fold higher in Ws. The results are from four to six biological treatments with three technical repeats for each measurement. Relative expression calculation and statistical analysis were carried out with REST 2009 software (Pfaffl *et al.* 2002). Asterixes above columns indicate significant differences between the mutants and the corresponding WT or treatments at $P < 0.05$ (*), $P < 0.01$ (**), and $P < 0,001$ (***) level according to t-test.

either a subtle defect in phyA signalling or a decreased phyB signalling output as argued by Liscum and Hangarter (1993).

A rather obvious feature of the mutant *pplaI-1* was the much higher tendency of the root coil formation on 45° tilted

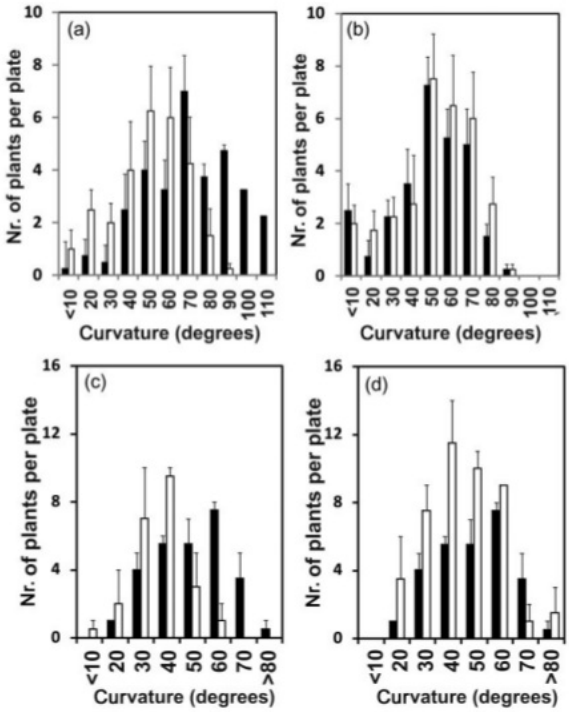

Figure 5. Phototropic bending of hypocotyls of 3-day-old dark-grown seedlings after 12 h lateral blue light (1 μE). Black bars: WT, white bars: mutants. (a) Ws: 67.1°, *ppla-I-1*: 45.6° ($P < 0.001$). (b) Col: 46.6°, *ppla-I-3*: 47.2°. (c) Col: 51.2°, *phyA:* 33.1° ($P < 0.001$). (d) Col: 51.2°, *phyB*: 41.2° ($P < 0.001$). (means indicated, n = 66–129).

mutants, FR light abrogated gravitropism *via* phyA therefore hypocotyls grew random (Fig. 7b). In fact, both *ppla-I* mutant lines showed a slightly weaker abrogation of hypocotyl gravitropism than the respective WT, while the *phyA* mutant, because blind to FR, grew almost upright (Fig. 7b). In this test, both *ppla-I* knockouts displayed phenotypes resembling

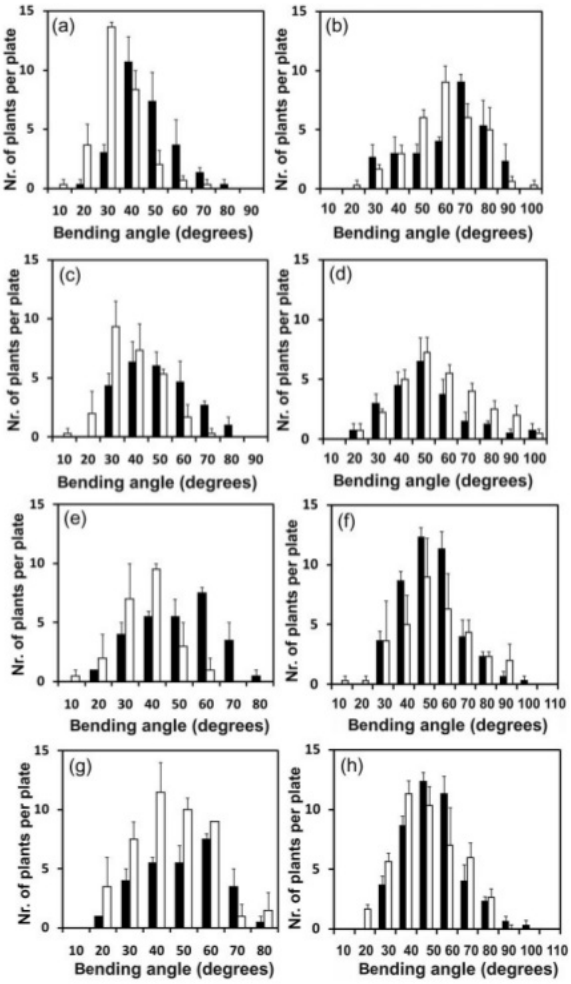

Figure 6. Gravitropic bending of hypocotyls or roots of 3-day-old dark-grown seedlings after 24 h tilting by 90°. (a,c,e,g) Hypocotyl bending angle frequencies. (b,d,f,h) Root bending angle frequencies. Black bars: WT. White bars: mutants. (a) Ws: 43.1°; *ppla-I-1*: 30.0° ($P < 0.001$). (c) Col: 45°; *ppla-I-3*: 33.8° ($P < 0.001$). (e) Col: 51.6°; *phyA*: 48.9°. (g) Col: 51.6°; *phyB*: 40.4° ($P < 0.001$). (b) Ws: 58.5°; *ppla-I-1*: 54.7°. (d) Col: 46.4°; *ppla-I-3*: 52.4° ($P < 0.014$). (f) Col: 54°; *phyA*: 54.9°. (h) Col: 54° *phyB*: 51.1°. Means indicated, $n = 73–120$.

hard agar plates (Simmons *et al*. 1995) than Ws WT's tendency (Fig. 7a,b,g). Here, the *ppla-I-3* line was indistinguishable from the Col WT (Fig. 8d,e,g), corresponding to results on root gravitropism. Coil formation was increased by increasing the osmotic strength of the medium (Fig. 8h). This has been noted before (Buer *et al.* 2000). When we tested *phyA* and *phyB* seedlings for root coil formation, *phyB* roots on 45° tilted agar produced about 80% coils, whereas *phyA* roots only produced 5–10% (Fig. 8c,f,i). To test contributions from blue-light receptors, we also investigated *cry1,2* and *phot1,2* double mutants. Both produced no coils (Fig. 8i).

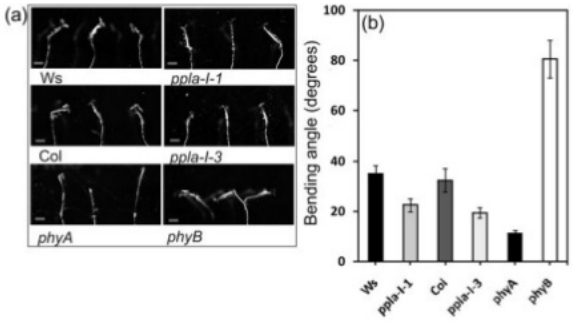

Figure 7. Interaction of far red light with hypocotyl gravitropism. Growth of seedlings in continuous far red light (10 μE) for 3 d and inhibition of gravitropism. (a) Representative seedlings of the different genotypes used. (b) The bending angle is the angle deviating from the plumb line in absolute values. In all treatments, the differences between respective wild type (WT) and *ppla-I* mutants were highly significant ($P < 0.001$; $n = 50–80$).

ppla-I mutants have a subtle light phenoytpe

We noticed that in short days, *ppla-I-1* flowered earlier. We verified this in a growth chamber under controlled conditions, but *ppla-I-3* did not flower earlier (Fig. 9a). Early flowering is consistent with reduced signalling by phyB or

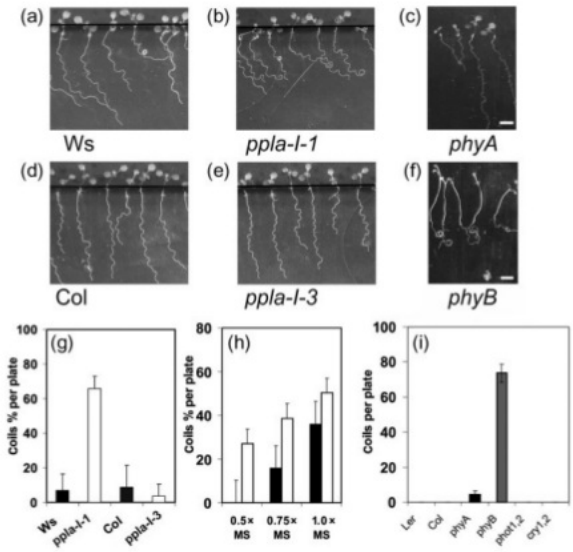

Figure 8. Formation root coils in seedlings grown on hard agar at a 45° tilted angle in (a,b,d,e) on 1/1 MS agar and in (c, f) on one-half MS agar. A plate with Ws wild type (WT) (a), *pplaI-1* (b), *phyA* (c), Col (d), *ppla-I-3* (e), and *phyB* in (f) is shown. (g) Comparison of coil frequency on 1/1 MS medium of the two mutant *ppla-I* lines. (h) Dependence of coiling response on the osmolarity of the medium in *ppla-I-1* (white bars) and Ws WT (black bars). (i) Comparison of coiling in of several photoreceptor mutants on one-half MS agar. Each experiment was conducted with four to six agar plates harbouring 15 or 30 seedlings each (SE).

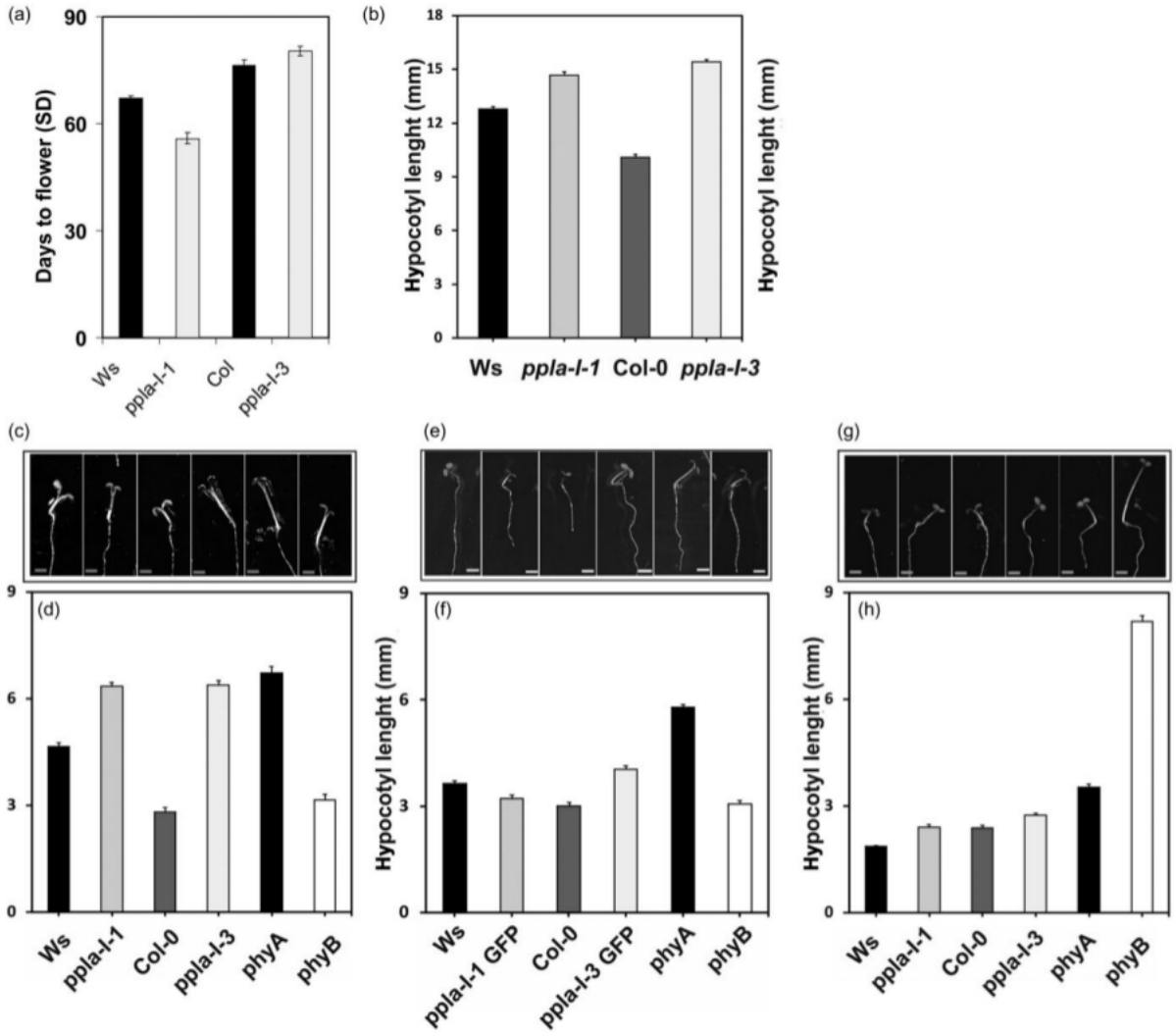

Figure 9. Phytochrome B-related properties in *ppla-I* null mutants. (a) Flowering dates of wild type (WT) and *ppla-I* mutants in short days (22 °C; 16 L/8D). The day of the appearance of the first flower was recorded. Differences between corresponding WT/ mutants were highly significant (both $P < 0.001$; $n = 36–39$; SE). (b–h) Light and shade avoidance response phenotype of *ppla-I* mutants. (b) Seedlings were grown for 3 d in the dark and then treated for 12 h with continuous far red and blue light (1 μE each). (c,d) Growth of seedlings in W supplemented with a low ratio of R:FR (red/far red). Bar = 2 mm. In (d) the differences between respective WTand *ppla-I* mutants were highly significant ($P < 0.001$). (e,f) WT and complemented seedlings grown in W supplemented with a low ratio R:FR light. Bar = 4 mm. (g,h) Growth of seedlings in W supplemented with a high ratio of R:FR (red/ far red). Bar = 2 mm; $n = 50–80$.

the phyB-like phyD missing in *ppla-I-1* (Devlin *et al*. 1999). Therefore, on our quest to understand the light phenotype in *ppla-I* mutants, we quantified the responses of the *ppla-I* mutant lines to continuous R, FR, and B (blue light) (Supporting Information Fig. S4). We could not find convincing differences between mutants and corresponding WT when grown in continuous monochromatic light (R, FR, B of 1 or 5 μmol m^{-2} s^{-1}). Only when we combined FR and B (1 μmol m^{-2} s^{-1} each) were both mutant alleles significantly taller than WT (Fig. 9b).

The combination of FR and B light could indicate a different response to shade just as early flowering. Therefore, we also tested the elongation response to W supplemented with low R:FR and high R:FR in hypocotyls of *ppla-I-1* and *ppla-I-3* and the respective WT and compared it with *phyA* and *phyB* mutants (Fig. 9c–h). In low R:FR conditions, both *ppla-I* mutant lines were much taller than the respective WT (Fig. 9c & d). Both WT were taller in low R:FR than in high R:FR, but the differences between *ppla-I-1* and Ws or *ppla-I-3* and Col in low R:FR were much greater. Therefore both mutants appear to be hypersensitive in their responses to low R:FR light, comparable with shade conditions. When we tested the *ppla-I* lines complemented by transformation of $P_{pPLA\text{-}I}$*:pPLA-I-GFP* the response to low ratio R/FR light

was like the respective WT (Fig. 9e & f). The classical explanation is that shade avoidance response to low R:FR is mediated by the low signalling output of phyB (Lau & Deng 2010; Stamm & Kumar 2010).

DISCUSSION

ppla-I-1 in Ws background and *ppla-I-3* in Col background show common phenotypes and background (accession)-specific characteristics

In regulation of early auxin genes, gravitropism of dark-grown seedlings, inhibition of hypocotyl gravitropism by FR light, and in response to FR-enriched W light, *ppla-I-1* and *ppla-I-3* are very similar. On the other hand, they differ in other responses (phototropism, early flowering, root coils). Several of these responses are known to involve phytochrome signalling or to potentially involve phytochrome signalling. In fact, Ws is a natural deletion mutant for *phyD* (Aukerman *et al.* 1997) and Col is not. We excluded differences in genomic and mRNA sequence and in RNA splicing as a basis for the phenotypic differences of the two lines (Fig. 1). Moreover, the response of both alleles to shade like conditions was complemented by expressing the pPLA-I-GFP protein in both alleles (Fig. 9g & h). The tissue distribution of *pPLA-I*-promoter-controlled GUS expression compared with the pPLA-I-GFP expression pattern also indicates that the pPLA-I protein is expressed in the same tissues, probably reconstituting function in the null background. Other gene differences besides *phyD* between Ws and Col are known (Ulm *et al.* 2001; Yang & Hua 2004; Bartels *et al.* 2009). Whereas the lack of *phyD* in *ppla-I-1* and Ws clearly seems to have an impact on the mutant phenotype, it remains elusive whether other genetic differences could have an impact on responses in *ppla-I* mutants.

ppla-I mutants have a dynamic auxin phenotype similar to *abp1* receptor mutants

Despite a lack of obvious morphological response to exogenous auxin in the knockout mutant lines compared with corresponding WT, we found clear defects in both knockout mutant lines in auxin-induced gene regulation using a set of selected genes as a physiological test (Fig. 4). This is reminiscent of our findings on the other investigated *abp1* and *eir1/pin2* mutants (Effendi & Scherer 2011; Effendi *et al.* 2011, 2013), and the other nine *ppla* null mutants that were tested the same way (Labusch *et al.* 2013). In *abp1/ABP1* seedlings, all auxin-regulated genes were mis-regulated while in *eir1/pin2*, 9 of 12 were affected. In the case of both *pplaI* alleles described here, 11 of 18 auxin-regulated genes were affected. Auxin-induced inhibition of *pPLA-I* transcription was minor after 30 min (Fig. 4e), so that transcriptional regulation of *pPLA-I* itself as a component of this signalling appears rather unlikely (Fig. 4d).

In contrast to the *pPLA-I* gene itself, whose expression was not changed within 10 min, we already found after 10 min, delayed transcription of early auxin-induced genes in *ppla-I* alleles (Fig. 4d). A transcriptional regulation by auxin of *pPLA-I* should be controlled by TIR1, but was not observed. Obviously, auxin-induced events elicited in less than 10 min need a receptor other than TIR1 (Scherer 2011; Scherer *et al.* 2012; Labusch *et al.* 2013). Therefore, our data rather indicate an auxin triggered post-transcriptional activation/inactivation event on pPLA-I to be evoked in less than 10 min. The strikingly similar phenotypes of *abp1/ABP1* and *abp1–5* (Effendi & Scherer 2011; Effendi *et al.* 2011, 2013) and the *ppla-I* alleles here, and the proven capacity of ABP1 to trigger rapid auxin responses (Scherer 2011; Scherer *et al.* 2012) favour ABP1 as the respective receptor although the specific mechanism remains to be uncovered. Moreover, the co-localization of pPLA-I (Fig. 3B, d–g; C, a,b,e,g) and ABP1 (Klode *et al.* 2011) in the plasma membrane, perinuclear ER and nuclear membrane is a precondition for their functional association.

Compromised phototropism, gravitropism and root coiling in *ppla-I* mutants point to pPLA-I functions in influencing PIN mobility and phytochrome signalling

Phototropism

Phototropin (PHOT) is the blue-light receptor-mediating phototropism in higher plants (Christie 2007). Few intermediates downstream from PHOT1 are known. Interactors with PHOT1 are NPH3 and PKS1. NPH3 binds to PHOT1 (Motchoulski & Liscum 1999; Pedmale & Liscum 2007), and PKS1 is one of four homologous proteins that act as intermediates between phototropin and phytochromes (Lariguet *et al.* 2006; Molas & Kiss 2008). Long known is the positive influence of red light on phototropism, and phyA was identified as the responsible phytochrome (Liu & Iino 1996a,b; Parks *et al.* 1996; Lariguet & Fankhauser 2004; Whippo & Hangarter 2004). A recent study describes a physical interaction between phyA and PHOT1 on the plasma membrane, making a modulation of directional sensing by phytochrome plausible and putting the receptors close to the auxin carriers (Jaedicke *et al.* 2012). Further downstream, PIN2 and PIN3 relocalization most likely mediates the auxin flux to the shaded flank in order to execute the asymmetrical growth response (Müller *et al.* 1998; Friml *et al.* 2002; Ding *et al.* 2011). Quite surprisingly, the phototropic defect in *ppla-I-1* was not found in *ppla-I-3*. Because one major difference between the Ws-derived mutant *ppla-I-1* and the Col-derived mutant *ppla-I-3* is the lack of *phyD* in *ppla-I-1* and Ws (Aukerman *et al.* 1997), this could change the interaction of intermediates common to the PHOT-induced and phy-induced pathways. Localization of pPLA-I near the plasma membrane would allow interaction with those (Fig. 3B, d). How exactly pPLA-I fits to these interactors remains to be solved.

Gravitropism

Gravitropic and phototropic bending involve differential growth regulation by auxin redistribution. Redistribution of

auxin transport is mainly dependent on mobile PIN proteins, more specifically PIN2 and PIN3. In darkness, defects in gravitropism in *pin2* and *pin3* originate mostly as a consequence of disturbed gravisensing and subsequent mis-regulation of PIN2 and PIN3 subcellular localization and hence, disturbed lateral auxin transport (Friml *et al.* 2002; Abas *et al.* 2006; Ding *et al.* 2011). Mis-regulation of early auxin-induced genes during the gravitropic response could be another reason for defects in gravitropic responses. Up-regulation of *PIN3* transcription by auxin was delayed in both mutant lines and may contribute to delayed gravitropism in our experiments. Other key auxin-dependent genes like *ARF7*, *IAA19* and *SHY2/IAA3* are supposed to be involved in hypocotyl gravitropism (Kim *et al.* 1998; Soh *et al.* 1999; Harper *et al.* 2000; Liscum & Reed 2002; Tatematsu *et al.* 2004). *PIN3*, *IAA19*, *IAA3* and *SAUR15* (Fig. 4a–d) were mis-regulated in *ppla-I-1* and *ppla-I-3*. These components have been implicated to regulate growth asymmetry in response to lateral auxin transport carried out by PIN2 and PIN3 (Friml 2003).

Gravitropism is abrogated by FR and R (Poppe *et al.* 1996; Robson & Smith 1996; Correll & Kiss 2005), and abrogation light is mediated by phyA and phyB (Fankhauser & Casal 2004). A delayed gravitropic response of hypocotyls grown in darkness was found in both *ppla-I* lines and in *phyB*, but to a much lower extent in *phyA*, meaning that in this experiment, gravitropism, was not influenced by light and yet *ppla-I* alleles responded in a manner similar to a *phyB* mutant. We point out, however, that in a *phyB* signalling mutant possessing no PIF1 or PIF3 transcription factors, gravitropic orientation is also disturbed in the dark (Shin *et al.* 2009). The same phenotype is much stronger in the quadruple loss-of-function mutant *pif1pif3pif4pif5* in both darkness and light (Kim *et al.* 2011). Further components downstream from phyB, which act in the regulation of hypocotyl orientation are GIL1 (Allen *et al.* 2006) and PKS4 (Schepens *et al.* 2008). Here both *ppla-I* alleles show a weak phenotype similar to the *phyA* mutant. This observation might indicate a role of pPLA-I in phyA signalling although low phyB signalling output cannot be excluded. As cytoplasmic phyA signalling has been described (Rösler *et al.* 2007, 2010) and an interaction of phyA and PHOT near the plasmamembrane has been observed (Jaedicke *et al.* 2012), a role for a PLA in modulating phyA signalling appears possible.

Coiling

Root coiling in the *ppla-I-1* mutant is likely to be caused by decreased gravisensing in the roots, which is a phenotype similar to the phenotype of several auxin-signalling mutants (Simmons *et al.* 1995; Sedbrook & Kaloriti 2008). Further factors contributing to root coiling are circumnutation and negative thigmotropism (Mullen *et al.* 1998; Migliaccio & Piconese 2001). Lack of *phyA* or *phyB* is known to decrease hypocotyl gravitropism (Robson & Smith 1996) and root gravitropism (Kunihiro *et al.* 2011), which explains why the *phyB* mutant strongly and the *phyA* mutant to a weaker degree produce root coils (Fig. 7). Measurements on the influence of light on auxin transport showed that in tomatoes, phyB2 exerts the strongest positive influence (Liu *et al.* 2011), supporting our observations that root coils in *ppla-I-1* could be a consequence of reduced auxin transport because of compromised phyB signalling in the roots. Because the additional *phyD* deletion in *ppla-I-1* may make it a weak *phyB/phyD*-like mutant the higher numbers of coils in *ppla-I-1* as compared with *ppla-I-3* could be a result of the missing phyD.

ppla-I mutants are hypersensitive to shade conditions

The most surprising aspect of the *ppla-I* mutant phenotype was the hypersensitive response to shade light. At the same time, this involvement of pPLA-I in the regulatory interaction of auxin and red light signalling, suggests this is starting with ABP1 as the receptor. The very same phenotypes in *abp1–5* and *abp1/ABP1*, and in *ppla-I*, together with all the other facets of auxin- and red light-related phenotype elements, provide this hypothesis (Scherer *et al.* 2012). Potential interactors of phototropism and gravitropism and red light were discussed earlier and may in part explain delayed tropistic responses.

Elongation of hypocotyls in shade is considered to be due to the down-regulation of phyB signal output as the major receptor for this response (Lau & Deng 2010; Stamm & Kumar 2010). Shade avoidance is somewhat enhanced in a *phyBphyD* double mutant, but also in a *phyD* single mutant (Devlin *et al.* 1999), showing that the two genes redundantly regulate this response. phyA has been shown to inhibit hypocotyl extension in light-grown plants and hence, contributes to plant reactions to changes in the R:FR ratio (Johnson *et al.* 1994). In low R:FR, the *phyA* mutant has long hypocotyls similar to those of *phyB* seedlings in high R:FR (Fig. 9). This reaction of *phyA* seedlings reflects the missing suppression of phyA on hypocotyl elongation in low R:FR shade (i.e. high FR) conditions in addition to low phyB signalling output. This indeed was also apparent in the mutant lines, and could be complemented by the expression of *pPLA-I:GFP* (Fig. 9c,d).

A HIR-insensitive or B-insensitive phenotype, as seen in *phyA* mutants, was not observed (Supporting Information Fig. S4). Only in a combination of FR and B, a hypocotyl growth inhibition was observed (Fig. 9b) supporting our interpretation along the classical views that shade-induced elongation is mainly due to decreased phyB output. The early-flowering phenotype of *pplaI-1* could be a result of a decreased output of phyB-like phytochromes in Ws because Ws is a *phyD* null mutant (Fig. 8a) (Devlin *et al.* 1999). Nevertheless, in view of some phyA-influenced phenotypes in the *ppla-I* mutants, a contribution of phyA-dependent HIR cannot be completely ruled out.

Hypersensitivity to shade conditions may explain why a low JA content in *ppla-I-1* and *ppla-I-2* has been found (Yang *et al.* 2007). Both their *ppla-I-1* and *ppla-I-2* alleles are in the Ws background. Moreover, the accumulation of jasmonate in response to *Botrytis* was indistinguishable in Ws and mutants

so that pPLA-I was concluded to not be the enzyme to release linolenic acid for jasmonate biosynthesis (Yang *et al.* 2007). Jasmonate is an inhibitor of elongation so that depending on the quality and quantity of light, the jasmonate content of plants defective in phyB signalling could be low (Robson *et al.* 2010). Consistent with this notion is the observation that the jasmonate receptor mutant *coi1–16* shows exaggerated sensitivity of hypocotyl elongation to shade (Robson *et al.* 2010). On that basis, our observations are consistent with the previous findings on pPLA-I functions in JA responses *ppla-I-1* (Yang *et al.* 2007).

The *ppla-I* and *abp1* mutants identify a function at the intersection of auxin and light signalling. There is overall similarity of phenotypes of the *abp1/ABP1* and *abp1–5* mutant and the *ppla-I* mutants, which consist of the similar regulatory defects in auxin-dependent responses like gene regulation, phototropism and gravitropism (Effendi & Scherer 2011; Effendi *et al.* 2011, 2013). Additionally, both *ppla-I-1* and *abp1/ABP1* flower early.

As a hypothetical mechanism, we suggested that ABP1 binds to a transmembrane protein as a co-receptor, likely a receptor kinase, which could transmit the auxin signal to a cytsolic network one component of which is suggested to be pPLA-I Effendi *et al.*, 2013). Recent publications described a mechanism to down-regulate phyB and phyB by phosphorylation in the cytosol (Medzihradszky *et al.* 2013; Nito *et al.* 2013). There is only one report that the fatty acid oleic acid activates a protein phosphatase and pPLA-I could provide such a metabolite (Baudouin *et al.* 1999). The results presented here are a platform for further research on this first PLA identified to have functions in light signalling. We therefore conclude that pPLA-I functions downstream of ABP1 and at so far unknown factors common to phytochromes and ABP1.

MATERIALS AND METHODS

Growth conditions and physiological experiments

For gravitropism and phototropism experiments, seeds were stratified for 4 d, treated for 4 h with W and grown for 3 d on upright 0.5× MS agar plates in the dark at 22.5 °C. For testing gravitropism, plants were turned by 90° for 24 h and then scanned; for phototropism 1 μE lateral B was applied and scanned after 8 h (CanoScan 8800F Canon Deutschland, Krefeld, Germany; resolution 600 dot per inch). For testing shade avoidance, after stratification seeds were treated with white light (W) for 4 h, and then kept in the dark for 24 h. For 3 subsequent days, W (24.5 μmole m^{-2} s^{-1}) was applied, after that red and FR either with a high R:FR ratio (2.1) or a low R:FR ratio (0.098) was applied in a light-emitting diode box at 22.5 °C (CLF, Plant Climatics, Wertingen, Germany) for another 3 d at 22.5 °C. Light-growth experiments with continuous light were carried out in a similar fashion. Lengths or angles were measured using AxioVision LE Ver.4.6 software (Zeiss, Oberkochen, Germany). For flowering experiments, plants grew in a growth chamber at 22.5 °C in 8/16 (L/D). For root coiling assays, seedlings were grown in the light on 2% hard agar containing half or full concentration of MS medium plus 1 or 2% sucrose, respectively (Murashige & Skoog 1962). Plates stood upright for 3 d to orient the roots according to gravity, then tilted to 45° to induce coiling (Simmons *et al.* 1995).

Identification of homozygous knockout mutant lines

Homozygous *AtplaI-1* knockout mutant plants in the Ws background were isolated as described (Krysan *et al.* 1996; see primer list). The site of T-DNA integration for *Atpla-1-1* was localized by sequencing of amplified PCR product in the second exon at nucleotide 22856893, corresponding to nucleotide 483 counted from the A in the AUG codon in the coding sequence and thus inserted into the second intron. Yang *et al.* (2007) isolated two knockout mutant alleles in the Ws background from the same collection (Krysan *et al.* 1996) and localized one insertion in exon2, but reported the insertion at nucleotide 438 after the AUG codon, which, however, corresponds with the end of the first exon (assuming the AUG is included into counting). However, in Fig. 1, the insertion is placed into in the second exon. Therefore, we conclude that their T-DNA insertion mutant, giving rise to their mutant *ppla-I-1*, is highly likely to be identical to the one we isolated (nucleotide 483 instead of 438), so it was also named *ppla-I-1*. Homozygous knockout *pplaI-3* plants (SALK_061667) in the Columbia background were isolated by using the same gene-specific primers described earlier plus the Lba1 primer and the T-DNA position for *pplaI-3* was 22856359 in the first intron. The lack of mRNA expression in the homozygous lines was shown by RT-PCR (see primer list).

P_{PLAI}::*uidA* construct

The promoter sequence of *AtPLAI* (P_{PLAI}) comprises 1940 bp in the 5′-region of *AtPLAI* (At1g61850) and ends at nucleotide +1 prior to the translation initiation site, including the unknown transcriptional start site. The primers (see list) included *Sal*I and *Xba*I restriction sites at their 5′ends, respectively. The promoter fragment was digested with *Sal*I and *Xba*I restriction enzymes and ligated in front of the *uidA* gene of the binary plant transformation vector pGPTV and introduced into the *Agrobacterium tumefaciens* strain GV3101. *Arabidopsis thaliana* (Col) were used for *in planta* transformation (Bechtold *et al.* 1993).

Histochemical GUS assay

Plants were grown on soil in the greenhouse to stain shoots, petioles and flowers, and grown *in vitro* on MS agar for staining roots and small leaves. To stain shoots, petioles and roots, the plant tissue was soaked in 2 mM 5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid (X-Gluc) in 50 mM NaH_2PO_4 (pH 7.0), 0.5% Triton-X, and 0.5 mM potassium ferri- and ferrocyanide for 24–48 h at 37 °C. To stain leaves and flowers, this substrate buffer containing 1 mM X-Gluc was used for 16–22 h at 37 °C (Jefferson 1987). Three different lines showed the same pattern of GUS staining.

Expression of $P_{pPLA\text{-}I}$:*pPLA-I-GFP* in *ppla-I* lines and confocal laser scan microscope

A 7.8-kb genomic fragment of DNA containing the coding region of *pPLA-I* (*At1g61850*) and 1.42 kb 5′- and 0.55 kb 3′-untranslated regions was amplified by PCR from BAC clone F8K4 and the cDNA sequence was verified. The construct was introduced into the *A. tumefaciens* strain GV3101 with the vector pGWB4 (Nakagawa *et al*. 2007) by heat shock transformation. Plant transformation was done into *pPLA-I* knockout mutant *ppla-I-1* using the vacuum infiltration method (Bechtold *et al*. 1993) to produce $P_{pPLA\text{-}I}$:*pPLA-I-GFP*. Seed selection was done by plant growth on 1/2 MS media containing hygromycin. Selected seedlings were cultivated in the greenhouse and seeds were harvested.

Seedlings of the transgenic line $P_{pPLA\text{-}I}$:*pPLA-I-GFP* with native promotor were grown for 7–28 d on 1/2 MS media, 1% agar on vertically plates and exposed to W (16/8 h). Seedlings were mounted in water, covered with glass slides and sealed using nail polish. Samples were visualized using an inverted confocal laser scanning Carl Zeiss LSM 510 Axiovert 200 M Zeiss microscope (Carl Zeiss, Inc., Oberkochen, Germany) with standard filter set. Laser monochromatic excitation light = 488 nm was obtained from argon/krypton gas mixture. Emission light was collected using a long-pass 505 nm filter for GFP signal. Image analysis was done with the Zeiss LSM software and ImageJ (Java-based image processing program provided freely by the National Institutes of Health).

Immunocytochemistry

For immunization, almost the complete C-terminal exon18 was cloned from the BAC clone CD4–16 into pDONR221 and then into expression vector pEX-2TM-GW expressed as his-tagged fusion protein and affinity-purified for immunization (Pineda Antikörper-Service, Berlin). Gateway primers are listed in Supporting Information Table S1. The antibody against the N-terminus was made against a synthetic peptide identified in the N-terminus (H_2N-CMQDEGNRSVIGKDEN-COOH) by Eurogentec (Aachen, Germany). Fixation and whole mount immunocytochemistry was made as described (Gälweiler *et al*. 1998).

Reporter construct

The promoter sequence of *AtPLAI* (P_{PLAI}) comprises 1940 bp in the 5′-region of *AtPLAI* (At1g61850) and ends at nucleotide +1 prior to the translation initiation site, including the unknown transcriptional start site. The primers (see list) included *Sal*I and *Xba*I restriction sites at their 5′ends, respectively. The promoter fragment was digested with *Sal*I and *Xba*I restriction enzymes and ligated in front of the *uidA* gene of the binary plant transformation vector pGPTV and introduced into the *A. tumefaciens* strain GV3101. *A. thaliana* (Col) was used for *in planta* transformation (Bechtold *et al*. 1993). For mesophyll protoplast transformation, cDNA of *AtPLA-I* was amplified with primers HH115 and HH116 and cloned into SalI and KpnI digested pGFP2 (Kost *et al*. 1998). Protoplast transformation and observation was essentially done as described in Rösler *et al*. (2007).

Transcriptional measurements

For transcription measurements of early auxin genes, seedlings were grown in MS/2 liquid medium for 7 d under long day conditions. For auxin treatment, the medium was removed and replaced with fresh medium. After 4 h of calibration in the fresh medium, seedlings were treated either with 10 μM IAA or only with MS/2 liquid medium for 30 min or as indicated. Seedlings were blotted on filter paper and frozen in liquid nitrogen for further use.

For quantitative RT-PCR, total RNA from auxin-treated seedlings was prepared using TRIzol® reagent according to the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA). RNA was converted to cDNA with RevertAid™ H Minus First Strand cDNA Synthesis kit (Fermentas, Vilnius, Lithuania). Primers were selected from previous works (Li *et al*. 2011; Effendi *et al*. 2011; see primer list). Relative expression calculation and statistical analysis were carried out with REST 2009 software (Livak & Schmittgen 2001; Pfaffl *et al*. 2002). The expression level for the control treatment was set as onefold. PCR conditions were: activation of the polymerase at 95 °C for 10 min; 40 cycles of DNA melting at 95 °C for 15 s and DNA annealing at 62 °C for 60 s. Each data point is the mean value of four to six biological treatments and three technical repeats for each of those.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Expression of $P_{pPLA\text{-}I}$*:pPLA-I-GFP* in *ppla-I-3* background.

Figure S2. Immunocytochemistry of tissue and subcellular localization of pPLA-I.

Figure S3. Immunocytochemistry in ppla-I-1 and ppla-I-3 mutants.

Figure S4. Responses to continous colored light in *ppla-I* mutants.

Table S1. Primer List.

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2	Steffen Rietz, Georgi Dermendjiev, Esther Oppermann, Fikadu Getah Tafesse et al. "Roles of Arabidopsis Patatin-Related Phospholipases A in Root Development Are Related to Auxin Responses and Phosphate Deficiency", Molecular Plant, 2010 Publication	1%
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