IPGSA CONFERENCE 2010

20th International Conference on Plant Growth Substances

28th June to 2nd July 2010
Tarragona (SPAIN)

I309

CERTIFICATE OF ATTENDANCE

We hereby certify that,

MR. YUNUS EFFENDI

has attended the

20th International Conference on Plant Growth Substances

held at the Universitat Rovira i Virgili, Tarragona (Spain), on 28th June to 2 July 2010.

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	June 28 (Monday)	June 29 (Tuesday)	June 30 (Wednesday)	July 1 (Thursday)	July (Friday)
09:00	Registration (opens every day)	**P2** Hormone Biosynthesis and Transport *Shinjiro Yamaguchi (Japan)*	**P3** Hormone Perception and Signaling *Joe Kieber (USA)*	**PS09** Evolution of Plant Hormone Signaling **PS10** Root Development **PS11** Abiotic Stress **PS12** Chemical Biology	**P4** Hormones and Environment *Salomé Prat (Spain)*
09;45		*Paul Staswick (USA)*	*Bonnie Bartel (USA)*		*Moto Ashikari (Japan)*
10:30		Coffee break	Coffee break	Coffee break	Coffee break
11:00		*Tetsuya Higashiyama (Japan)*	*Zhiyong Wang (USA)*	**PS09** Evolution of Plant Hormone Signaling **PS10** Root Development **PS11** Abiotic Stress **PS12** Chemical Biology	Steve Penfield (UK)
11:30	Opening				
11:45	Silver Medal Lectures *Joanne Chory (USA)*	*Jiri Friml (Belgium)*	*Erwin Grill (Germany)*		*Luis Herrera-Estrella (Mexico)*
12:30	*Tokao Yolota (Japan)*	Lunch & Posters	Lunch & Posters	Lunch & Posters	Closing
13:00					Farewell Lunch
13:15	*Mark Estelle (USA)*				
14:00	Lunch				
15:00	**P1** Hormones and Development *Ottoline Leyser (UK)*	**PS01** Hormone Biosynthesis **PS02** Seed Development and Germination **PS03** Auxin Transport **PS04** Light Responses	**PS05** Hormone Perception and Signaling **PS06** Shoot Development **PS07** Defense responses **PS08** Systems Biology	**PS13** Hormone Interactions **PS14** Reproductive Development **PS15** Hormones and Biotechnology **PS16** Epigenetics/Small RNAs	
15:45	*Eliezer Lifschitz (Israel)*				
16:30	Coffee break	Coffee break	Coffee break	Coffee break	
17:00	*Markus Grebe (Sweden)*	**PS01** Hormone Biosynthesis **PS02** Seed Development and Germination **PS03** Auxin Transport **PS04** Light Responses	**PS05** Hormone Perception and Signaling **PS06** Shoot Development **PS07** Defense responses **PS08** Systems Biology	**PS13** Hormone Interactions **PS14** Reproductive Development **PS15** Hormones and Biotechnology **PS16** Epigenetics/Small RNAs	
17:45	*Veronica Grieneisen (UK)*				
18:30	Wellcome Reception	Tarragona Roman Ruins visit		IPGSA Business Meeting	
19:30					
20:00			congress Dinner		

PS05-18 **STRUCTURAL BASIS FOR GIBBERELLIN RECOGNITION BY ITS RECEPTOR GID1**
Miyako Ueguchi-Tanaka (Japan)

PS05-19 **MIS-REGULATION OF EARLY AUXIN-INDUCED GENES IN PHOSPHOLIPASE A KNOCKOUTS**
Corinna Labusch (Germany)

PS05-20 **CHEMICAL SCREENING AND CHARACTERIZATION OF INHIBITORS FOR GIBBERELLIN RECEPTOR**
Jung-min Yoon (Japan)

PS05-21 **A POSSIBLE CYTOSOLIC ACTIVITY FOR THE GA RECEPTOR, GID1**
Sivan Sagiv (Israel)

PS05-22 **DOMAIN REQUIREMENTS FOR DIFFERENT ROLES OF THE ETR1 ETHYLENE RECEPTOR**
Brad Binder (USA)

PS05-23 **TRANSCRIPT PROFILING OF CYTOKININ ACTION IN ARABIDOPSIS ROOTS AND SHOOTS**
Brenner Wolfram (Germany)

PS05-24 **THE IMA GENE ENCODES AN ADAPTER MINI ZINC FINGER PROTEIN REGULATING THE DEVELOPMENT OF FLORAL MERISTEM IN TOMATO.**
LEBLOND JULIE (FRANCE)

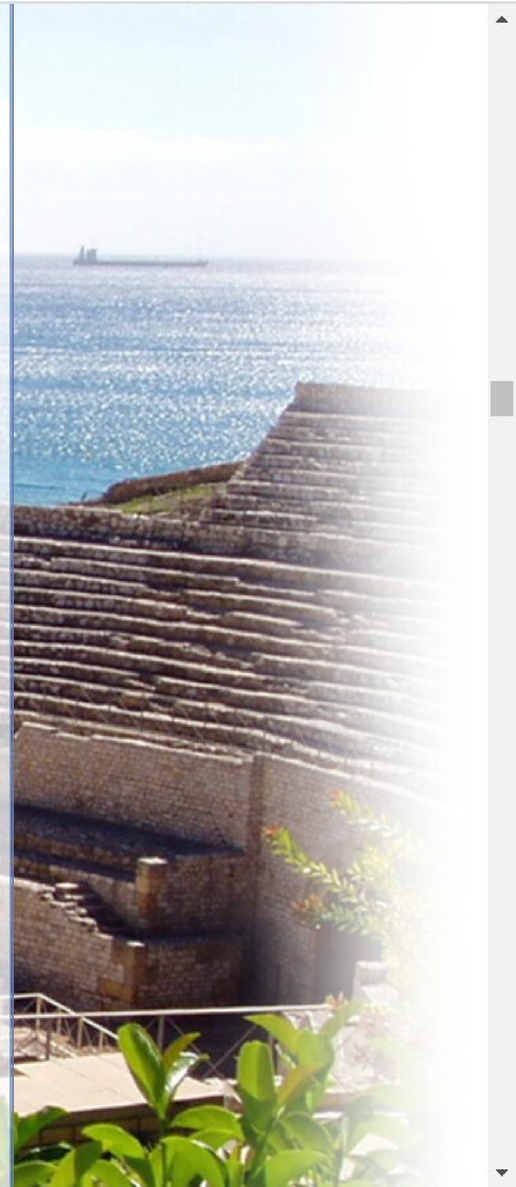
PS05-25 **QUANTITATIVE DIFFERENTIAL ANALYSIS OF PLANT PROTEOME IN A RESPONSE TO HORMONAL REGULATIONS**
Marketa Zdarska (Czech Republic)

PS05-26 **DYNAMIC STRUCTURE AND BINDING SPECIFICITY OF THE RECEIVER DOMAIN OF SENSOR HISTIDINE KINASE CKI1**
Tomas Klumpler (Czech Republic)

PS05-27 **ATPLA-I, A PHOSPHOLIPASE A WITH FUNCTIONS IN GRAVITROPISM, PHOTOTROPISM AND ROOT TIP MOVEMENTS**
Günther Scherer F.E. (Germany)

PS05-28 **AUXIN-BINDING PROTEIN1 (ABP1), THE SECOND AUXIN RECEPTOR**
Yunus Effendi (Germany)

PS05-29 **MUTATION OF TRNA SPECIFIC ISOPENTENYLTRANSFERASES AFFECTS ARABIDOPSIS PHENOTYPE**
Silvia Gajdosova (Czech Republic)



MIS-REGULATION OF EARLY AUXIN-INDUCED GENES IN PHOSPHOLIPASE A KNOCKOUTS

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Abstract

Patatin-related phospholipase A are coded by ten genes (AtPLAs) in Arabdipsis thaliana and are involved in auxin and pathogen signaling (e.g. Rietz et al., 2010, Mol. Plant). Here we used the T-DNA insertion mutants of the AtPLAIIIA, AtPLAIVB, AtPLAIVC, AtPLAIVD and AtPLAV to test the regulation of early auxin genes. Test genes were IAA2, IAA11, IAA13, IAA20, SAUR9, SAUR15, SAUR23, GH3.5 and genes involved in lateral root formation (IAA1, IAA4, IAA9, PIN3). 30 to 60% of the genes tested failed to respond to auxin (1 µM/10 µM) at t=30 min in the different knockouts. In most mutants the genes IAA11, IAA13 and IAA20 showed no change in gene expression in comparison to the WT, except IAA2 in AtPLAVB and AtPLAVD. Many of the genes involved in lateral root formation and the SAUR genes showed s atrong defect in gene expression in the mutants after auxin application. The mutants did not show any phenotypes under normal growth conditions. Only AtPLAIVA showed 50% lateral root formation on low nutrient medium. AtPLAIVC reacted less sensitive to ABA and to Phosphate deficiency (Riets et al, 2010). Because the abp1/ABP1 mutant regulated none of early auxin-induced genes properly at 30 min, we hypothesize that ABP1 and PLAs act in the same auxin signaling pathway influencing TIR1 activity in an unknown way (FEBS Lett 581: 4205-4211)

Keyword: Phospholipase A, AtPLA, early auxin-induced genes

Introduction

Phospholipase A (PLA) hydrolyses phospholipids either at the hydroxyl group of the C1 (PLA1) or of the C2 atom (PLA2) 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 PLA2 (sPLA2) (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 PLA2 (iPLA2) in animals (Six & Dennis 2000; Balsinde & Balboa 2005) and patatin-related PLA (pPLA) (Scherer et al. 2010). pPLAs hydrolyse fatty acids of both phosphoand 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 Gprotein-binding motif within this LRR domain, and a Cterminal domain with unknown function. Gene pPLA-I is the one gene most similar to the homologous so-called calciumindependent 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 auxinactivated 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 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

structural difference in gene or mRNAs of ppla-I-1 dan ppla-I-3

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 missplicing, 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.

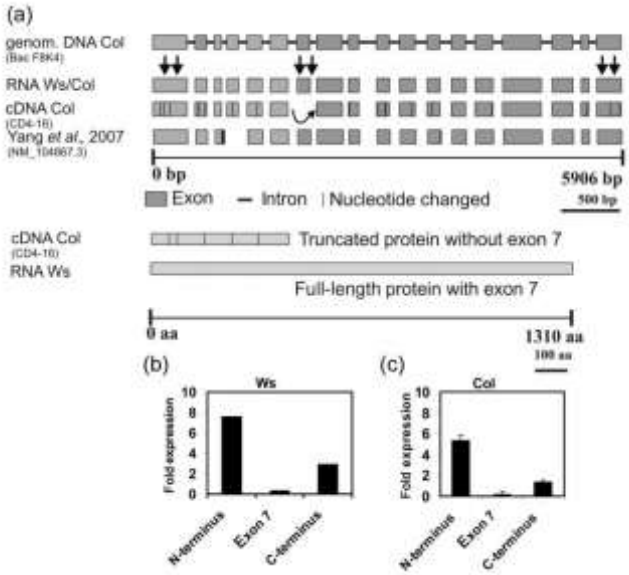


Fig. 1. Genomic and exon–intron structure, mRNA sequences and splicing and transcription of pPLA-I. (a) Genomic sequence, known and postulated RNA sequences, and deduced protein sequences. A genomic BAC clone [BAC F8K4, source Col wild type (WT)] was used to isolate large fragments, which were sequenced 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.

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 pplaI-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. 2). 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. 2a); SAUR genes (Fig. 2b); and PIN2, PIN3, and GH3.5 (Fig. 2c). Thirty minutes after the application of 10 uM, 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.

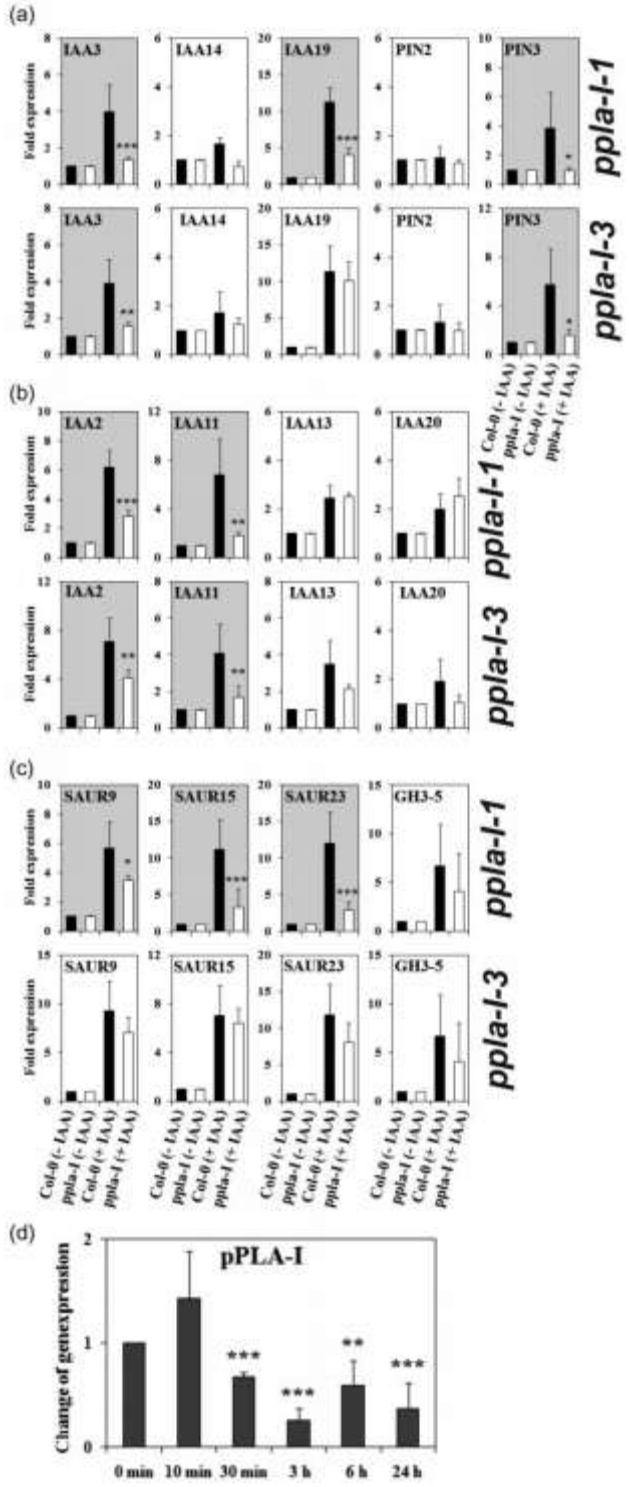


Fig. 2. Regulation of early auxin-inducible genes in light-grown ppla-I-1 and ppla-I-3 knockout mutant lines and respective WT seedlings. The results are arranged into different groups of early auxin-inducible genes and other genes. (a) Regulation of selected IAA genes. (b) Transcription of SAUR genes (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 uM 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.

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 inducible genes.

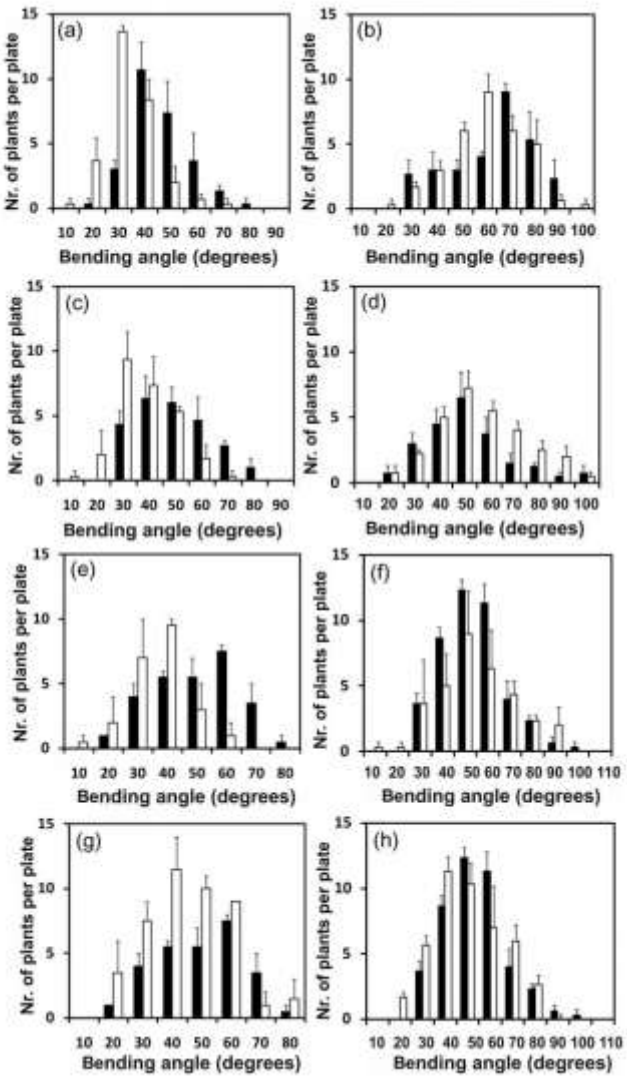
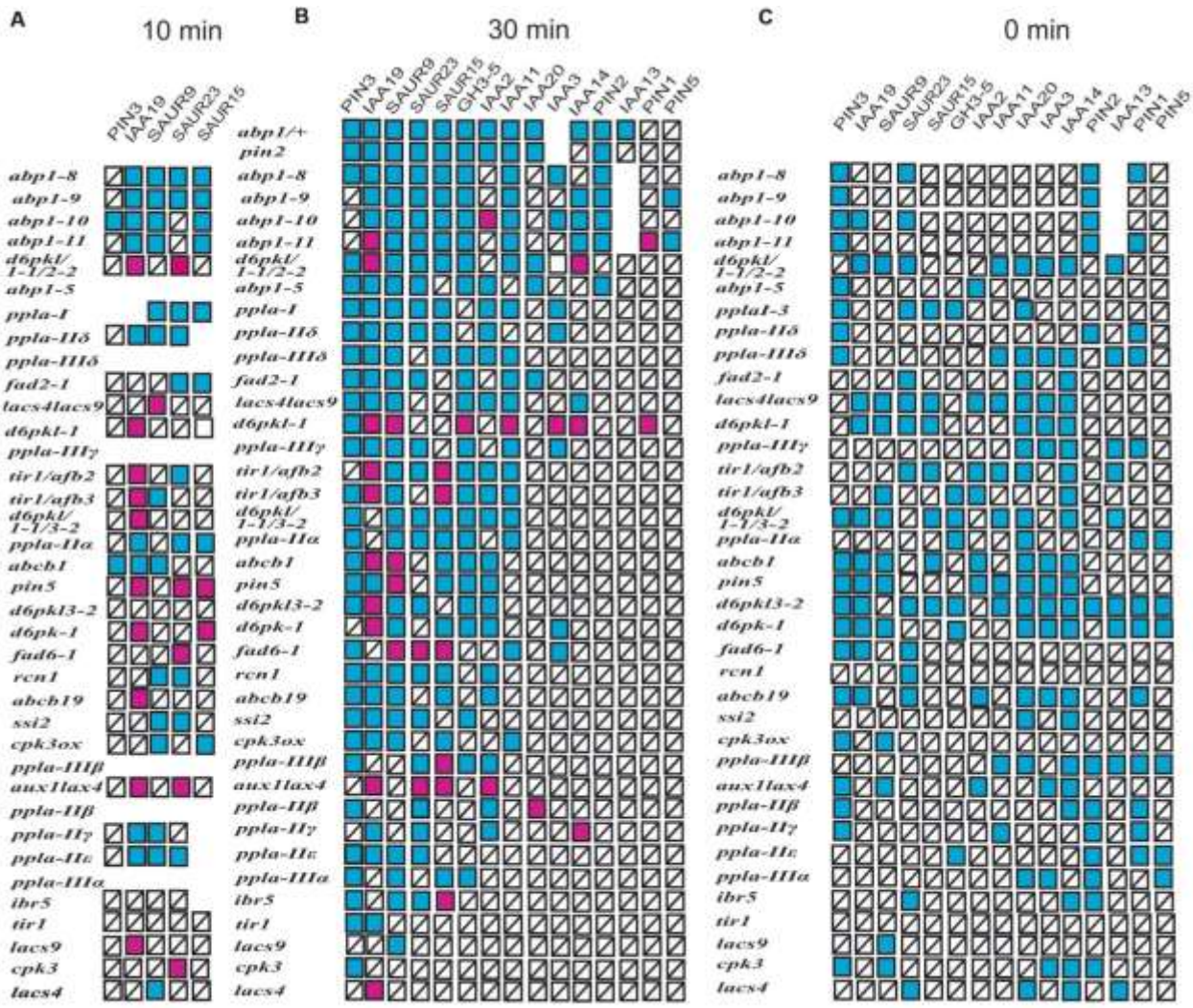


Fig. 3. Gravitropic bending of hypocotyls or roots of 3-day-old dark-grown seedlings after 24 h tilting by 90°. (a,c,e,g) Hypocotyl bending angle frequencies. (b,d,f,h) Root bending angle frequencies. Black bars: WT. White bars: mutants. (a) Ws: 43.1°; ppla-I-1: 30.0° ($P < 0.001$). (c) Col: 45°; ppla-I-3: 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.

Comparison of Long-Term Expression of Reporter Genes to their Short Term Induction by Auxin

To allow a synopsis and a comparative analysis, we assembled all previously published results on ppla and abp1 mutants (Effendi et al., 2013, 2014, 2015; Labusch et al., 2013) and the experiments here into one simplifying scheme (Figure 4) of the mis-expression of the reporter genes after 10 (Figure 4A) and 30 min of 10 mM auxin treatment (Figure 4B) and at t = 0 min (Figure 4C). The subcellular localization of the mutant gene products is summarized in Figure 4D. Previously unpublished data on ppla mutants at t = 0 are incorporated into Figure 4C. The mutant lines are

arranged vertically according to the number of mis-regulated reporter genes after 30 min of auxin application (Figure 4B), and the list of reporter genes is arranged with the most frequently misregulated reporter genes on the left side. In the scheme for t = 10 min (Figure 4A) and t = 0 min (Figure 4C) the genes were arranged accordingly. In the pplaI mutant, we had found eight mis-regulated reporter genes (Labusch et al., 2013; Effendi et al., 2014) and in the other ppla mutants four to seven at t = 30 min (Labusch et al., 2013; Effendi et al., 2014). All abp1 mutants had high scores of mis-regulated reporter genes at t = 30 min (8 in abp1–5 and 9–12 in the engineered abp1 mutants at t = 30 min). At t = 10 the mutants abp1–8, abp1– 9, abp1–10, and abp1–11 transcribed the reporter genes generally at a lower rate (Figure 4B; and data from Effendi et al., 2015). As stated before, the only weak mutants in our auxin-induced transcription test (t = 30 min) were tir1, lacs4, lacs8, and cpk3, probably because all of them are members of gene families so that genetic redundancy could play a role to reduce the impact of a single gene mutant.


A
10 min
B
30 min
C
0 min
PIN3
IAA19
SAUR9
SAUR23
SAUR15
GH3-5
IAA2
IAA11
IAA20
IAA3
IAA14
PIN2
IAA13
PIN1
PIN5
abp1/+
pin2
abp1-8
abp1-9
abp1-10
abp1-11
d6pkl/ 1-1/2-2
abp1-5
ppla-I
ppla-IIδ
ppla-IIIδ
fad2-1
lacs4lacs9
d6pkl-1
ppla-IIIγ
tir1/afb2
tir1/afb3
d6pkl/ 1-1/3-2
ppla-IIα
abcb1
pin5
d6pkl3-2
d6pk-1
fad6-1
rcn1
abcb19
ssi2
cpk3ox
ppla-IIIβ
aux1lax4
ppla-IIβ
ppla-IIγ
ppla-IIε
ppla-IIIα
ibr5
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lacs9
cpk3
lacs4

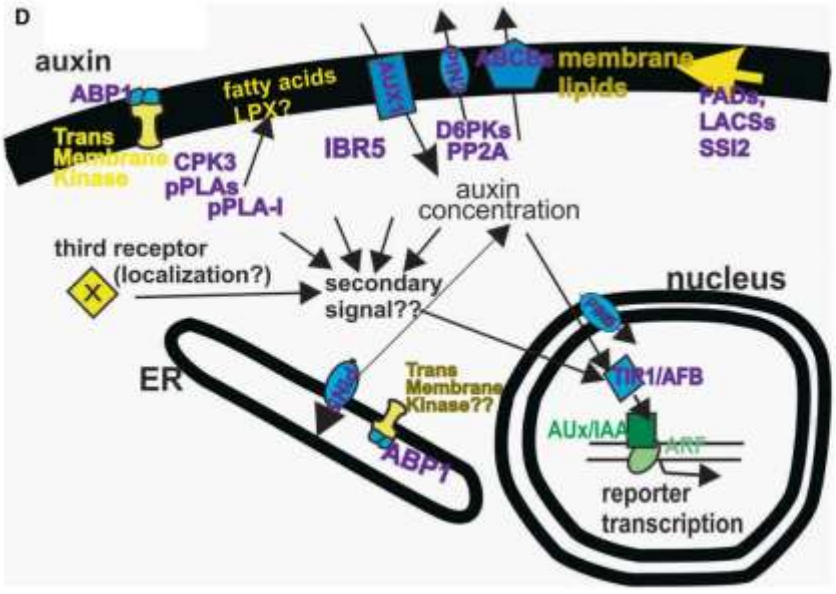
D
auxin
ABP1
fatty acids
LPX?
membrane
lipids
FADs,
LACSs
SSI2
Trans
Membrane
Kinase
CPK3
pPLAs
pPLA-I
IBR5
D6PKs
PP2A
auxin
concentration
third receptor
(localization?)
secondary
signal??
nucleus
ER
Trans
Membrane
Kinase??
TIR1/AFB
AUx/IAA
ARF
ABP1
reporter
transcription

Fig. 4. Summary of auxin-induced transcription in mutants and the number of defects in early auxin-induced gene expression. Red squares represent increased transcription as compared to the wt and blue squares represent decreased values than those found in the wt. Squares with a black bar represent transcription not significantly different from the wt

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 darkgrown 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-IGFP 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. 2). 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. 2e), so that transcriptional regulation of pPLA-I itself as a component of this signalling appears rather unlikely (Fig. 2d). 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.

Transcriptional Readout Controlled by the TIR1/AFB Receptors Is an Auxin Response

Transcription of early auxin-induced genes is a valid auxin response. The reporter genes, we chose clearly displayed a regulatory pattern. Some genes such as PIN1, PIN2, and PIN5 are non-responsive, others like IAA19, SAUR9, SAUR15, and SAUR25 are highly and rapidly responsive (4A,B) so that, as a whole, a clear pattern of fast responsiveness in the wt and in the mutants becomes apparent. This pattern of responsiveness is not identical to the pattern generated from transcription of the reporter genes at t = 0, which corresponds to the changes in gene expression during 7 days seedling development (Figure 4C). It should be noted that already the mis-regulation of only one reporter gene in only one mutant would mean that, we would have to conclude that an auxin receptor-driven process depending on this mutant gene product causes this. In fact, we found several mis-regulated genes in many mutants. Hence, deviations in single values do not undermine this hypothesis, the complete pattern is the true argument for it. The measurements at t = 30 min argue for a receptor-driven pathway to regulate TIR1/AFB activities with special emphasis for all mutant gene products which are membrane proteins (Figure 4D). This is given by the fact that membrane protein amounts are not rapidly changed by transcription/translation. Their expression at the plasma membrane needs 1 h or longer (Scherer, 2011). Changes in transcription of ABP1, PIN2, PIN3, ABCB1, ABCB19, and AUX1/LAX3 cannot affect expression at the plasma membrane within 30 min to cause back-coupling to TIR1. Similarly, it seems very unlikely that changes in membrane lipid composition will be measurable after 30 min transcription of genes of reporter genes in LACS4, LACS9, SSI2, FAD2-1, or FAD6-1 mutants. Lipid compositions are the target of these genes so that their changes could result in activity changes of membrane proteins, e.g., transport proteins which are influenced by lipid composition so that timing, either 10 min or 30 min auxin treatment, is not the point (Roudier et al., 2010; Markham et al., 2011).

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 UE 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 Umole 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.

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 M 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 RevertAidTM 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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