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阿拉伯芥中 FIN219 基因的功能性研究(1/3)

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阿拉伯芥中 FIN219 基因的功能性研究(1/3)

(Functional Studies of the FIN219 Gene in *Arabidopsis*)

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中文摘要：

FIN219 已被證實參與阿拉伯芥中光敏素 A 的訊息傳遞，並且是 COP1 的一個基因外抑制體。為了進一步的瞭解 FIN219 在植物發育過程與光訊息傳導過程中所扮演的角色，我們利用 yeast two-hybrid 的方法篩選可與 FIN219 相互作用的蛋白質。一個產生 GST （glutathione-S-transferase） 蛋白的基因 *FIP1*（FIN219-interacting protein 1）已被分離，並且它所產生的蛋白質被證實可與 FIN219 的 C 端作用。大量表現 *FIP1* 基因於 Columbia 野生型時，它的轉殖株表現出對遠紅光、紅光不敏感的長下胚軸外表型，而此外表型受 FIN219 的調控；但抑制 *FIP1* 表現的轉殖株則表現出相反的外表型，此結果暗示 FIP1 很可能是一個負調控者，參與遠紅光對下胚軸延長的抑制性。另外，Northern blot 分析的結果顯示 *FIP1* 基因可受白光的誘導，並且表現於遠紅光與藍光，但在黑暗與紅光中未檢測出它的表現。除此之外，它的 mRNA 表現在遠紅光下，在光敏素 A 與 *fin219*、*fhy3*、*cop1* 與 *hy5* 突變體中亦降低；而且在紅光下也受 *phyB* 與 *fhy1* 突變體的影響而降低。而 FIP1 的 mRNA 的表現亦受 auxin、jasmonate 與殺草藥的保護劑的誘導。利用洋蔥表皮細胞的 transient assay 中發現 *GUS* 與 *FIP1* 的轉譯融合蛋白同時位於細胞核與細胞質中，且光並不會改變它在細胞內的位置。綜合以上的結果顯示 FIP1 是一個可與 FIN219 作用，且參與光訊息傳遞，特別是遠紅光、紅光，與多個荷爾蒙訊息傳遞的溝通者。

關鍵詞：FIN219；FIP1(FIN219-interacting protein 1)；
GST(glutathione-S-transferase)；光敏素 A、B；茉莉酸（jasmonate）

英文摘要：

FIN219 has been shown to be involved in phytochrome A-mediated signaling pathway and acts as a suppressor of COP1 in *Arabidopsis*. To further understand the function of FIN219 in light signaling during *Arabidopsis* development, a yeast two-hybrid approach was utilized to isolate FIN219-interacting partners. A gene *FIP1* (*F*IN219-*i*nteracting *p*rotein1) encoding a GST(glutathione S-transferase) was recovered and demonstrated to interact with the C-terminus of FIN219. The transgenic plants overexpressing the *FIP1* gene in wild type Columbia display a hyposensitive phenotype with longer hypocotyls under continuous far-red and red light and this longer hypocotyl phenotype is dependent on the presence of the functional FIN219; however, *FIP1* antisense lines show an opposite phenotype, suggesting that FIP1 may act as a negative regulator for FR-mediated inhibition of hypocotyl elongation. Furthermore, the results of Northern blot analyses indicated that *FIP1* gene expression is upregulated by white light and detectable in FR as well as B light, but its expression is hardly detected in both dark and red light condition. Besides, its expression is reduced in phytochrome A mutants and several light signaling transducer such as *fin219*, *fhy3*, *cop1* and *hy5* mutants in continuous far red light condition and also decreased in *phyB* and *fhy1* mutants in continuous R light. Furthermore, *FIP1* expression is also induced by auxin, jasmonate and herbicide safeners. The GUS-FIP1 fusion protein is localized in both nucleus and cytoplasm based on the result of transient assays in onion cells. Taking all current results together, FIP1 is interacting with FIN219 and acts as a crosstalk between light signaling and phytohormone signaling.

Key words:
FIN219；FIP1(FIN219-interacting protein 1)；GST(glutathione-S-transferase)；Phytochrome A；Jasmonate

FIP1, a FIN219-interacting protein, is a glutathione S-transferase and involved in phytochrome A-mediated signaling pathway in *Arabidopsis*

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Summary

FIN219 has been shown to be involved in phytochrome A-mediated signaling pathway and acts as a suppressor of COP1 in *Arabidopsis*. To further understand the function of FIN219 in light signaling during *Arabidopsis* development, a yeast two-hybrid approach was utilized to isolate FIN219-interacting partners. A gene *FIP1*(*F*IN219-*i*nteracting *p*rotein1) encoding a GST(glutathione S-transferase) was recovered and demonstrated to interact with the C-terminus of FIN219. The transgenic plants overexpressing the *FIP1* gene in wild type Columbia display a hyposensitive phenotype with longer hypocotyls under continuous far-red and red light and this longer hypocotyl phenotype is dependent on the presence of the functional FIN219; however, *FIP1* antisense lines show an opposite phenotype, suggesting that FIP1 may act as a negative regulator for FR-mediated inhibition of hypocotyl elongation. Furthermore, the results of Northern blot analyses indicated that *FIP1* gene expression is upregulated by white light and detectable in FR as well as B light, but its expression is hardly detected in both dark and red light condition. Besides, its expression is reduced in phytochrome A mutants and several light signaling transducer such as *fin219*, *fhy3*, *cop1* and *hy5* mutants in continuous far red light condition and also decreased in *phyB* and *fhy1* mutants in continuous R light. Furthermore, *FIP1* expression is also induced by auxin, jasmonate and herbicide safeners. The GUS-FIP1 fusion protein is localized in both nucleus and cytoplasm based on the result of transient assays in onion cells. Taking all current results together, FIP1 is interacting with FIN219 and acts as a crosstalk between light signaling and phytohormone signaling.

INTRODUCTION

Light has a profound effect on plant growth and development. It not only provides energy source for plant photosynthesis, but also acts as an important signal to regulate gene expression and various aspects of plant development. Plants are equipped with different photoreceptors to sense the changes of light environment (Kendrick and Kronenberg, 1994). Recent progress on the research of light signal transductions by molecular genetics, cell biology and DNA microarray approaches has been greatly impressed (Liscum et al., 2003; Parks, B.M., 2003; Matsushita et al., 2003; Jiao et al., 2003; Tepperman et al., 2004; Bauer et al., 2004; Tepperman et al., 2001). Especially, phytochrome A-mediated signaling pathway in continuous far red has been extensively studied. Lots of intermediate transducers were isolated including FHY1/PAT3, FHY3, FIN2, FIN219, EID1, FAR1, PAT1, LAF1, SPA1 and RSF1/HFR1/REP1 (Desnos et al. 2001/Zeidler et al. 2001; Wang and Deng 2002; Soh et al. 1998; Hsieh et al. 2000; Dieterle et al. 2001; Hudson et al. 1999; Bolle et al. 2000; Ballesteros et al. 2001; Hoecker et al. 1999; Fankhauser and chory 2000/Fairchild et al. 2000/Soh et al. 2000). All these genes have been cloned and characterized at the molecular level except for *FIN2*. Since light can modulate subcellular targets of phytochrome A(phyA) (Kircher et al., 2002), all of the transducers involved in phyA-mediated signaling pathway were found to be localized in cytosol, for instance: FIN219 (Hsieh et al., 2000) and PAT1 (Bolle et al., 2000), or in nuclei such as FHY3, FAR1, LAF1, SPA1, EID1 and RSF1/HFR1/REP1, or in both like in the case of FHY1/PAT3 (Desnos et al. 2001/Zeidler et al. 2001). So phyA can transduce the light signal through these components or directly interact with transcription factor such as PIF3 to turn on gene expression (Martinez-Garcia *et al.*, 2000). In addition, recent evidence indicated that phyA-mediated signaling is desensinized through the ubiquitination of downstream positive regulators LAF1 and HY5 by COP1 (Seo et al., 2003; Hardtke et al., 2000), a key repressor of photomorphognesis in Arabidopsis. Furthermore, SPA1, a nuclear-localized suppressor of phytochrome activity, can modulate the expression levels of both positive regulators via interaction with COP1, thus desensitizing phyA-mediated signaling pathway (Saijo et al., 2003; Seo et al., 2003).

fin219 (far-red insensitive 219) mutant was derived from the extragenic suppressors screening of the *cop1-6* mutant in *Arabidopsis* and exhibited less sensitivity specifically to continuous FR(cFR) with long hypocotyl phenotype. Its gene was cloned by map-based method and derived product shares 36-47% identity with a GH3 gene family that is made up of 23 members in *Arabidopsis* genome (Hsieh et al.,

2000). GH3 was originally isolated from soybeans as a rapid induction by auxin (Hagen et al., 1984) and its function was believed to be involved in cell elongation (Nakazawa et al., 2001). FIN219 was also induced rapidly by auxin and localized constitutively in cytosol without changes of subcellular locations by light. In addition, FIN219 has been demonstrated indeed to be a suppressor of COP1 (Hsieh et al., 2000). However, its physiological function involved in light signaling and plant development remains to be solved. To further understand the function of FIN219 in *Arabidopsis*, a yeast two-hybrid approach was employed to isolate FIN219-interacting partners. One of three candidates was further characterized and reported here. This gene encoding a glutathione S-transferase (GST, At1g78370/AtGSTU20) was named FIP1(FIN219-interacting protein1) and demonstrated to interact with the C-terminus of FIN219. The transgenic plants overexpressing FIP1 in wild type Columbia displayed a hyposensitive phenotype with longer hypocotyls compared to that in wild type under cFR. However, transgenic plants with the antisense construct of FIP1 show an opposite effect, indicating that FIP1may function as a negative regulator for phyA-mediated signal transduction.

Isolation of FIN219-interacting Partners by Yeast Two-hybrid Method

FIN219 is involved in phyA-mediated light signaling pathway and induced rapidly by auxin. It shares 36-47% identity with GH3 members at amino acid level and contains two coiled-coil domains, one in the N-terminus and the other in the C-terminus (Figure 1A), which implies that FIN219 may interact with other proteins via these domains. To further understand the FIN219 function in light signaling and plant development, a yeast two-hybrid method was employed to isolate FIN219-interacting partners. FIN219 full length cDNA was cloned into a yeast GAL4 DNA-binding domain vector pGBT9 (+2) and used as a bait to screen a library CD4-10 from Arabidopsis Biological Research Center (ABRC). Three candidates were recovered and further checked by the use of yeast mating approach. One of them, named FIP1 (FIN219-interacting protein1), was characterized to show β-galactosidase activity and to interact with the C-terminal region of FIN219(Figure 1B). The interaction between FIP1 and FIN219 was further confirmed by pull-down assay (Figure 1C). Taking these interaction studies together indicated that FIP1 is indeed interacting with the C-terminus of FIN219.

FIP1 Encodes a Plant Glutathione S-transferase

FIP1 isolated from yeast two-hybrid screening was subjected for sequencing and

BLAST search, the result indicated that it encodes a plant GST with 217 amino acids (Glutathione S-transferase, AtGSTU20/At1g78370), which belongs to the large tau class of GST gene family in *Arabidopsis*. It shares a 67% identity at amino-acid level with At1g78340 (AtGSTU22) within the same tau class. The alignment of these two proteins was shown in figure 2. However, the similarity of FIP1 to other GSTs of different classes was below 25%. All plant GSTs display a very conserved structure although primary amino acid sequences are quite diverse, indicating that binding of the ligand like glutathione is important for their activity and function over evolution.

Transgenic Plants Overexpressing the FIP1 cDNA in Wild Type Exhibit a Hyposensitive Phenotype

In an attempt to determine if the FIP1 is involved in light signaling pathway, we overexpressed the FIP1 full-length cDNA as a *35S::cmyc::FIP1* construct into wild type Columbia to examine any effect on the phenotype. The results indicated that twelve out of 30 T2 transgenic lines showed a hyposensitive phenotype in continuous FR(cFR), in which the hypocotyl length is intermediate compared to that of wild type Columbia and *fin219* mutant(data not shown). In addition, we also established a translational fusion construct, 35S::pRTL2-GUS:: FIP1, and then overexpressed in wild type Columbia. 7 out of 14 T2 transgenic lines display the same phenotype as those containing the *35S::cmyc::FIP1* construct that are longer hypocotyls than that of wild type under cFR(figure 3A). Furthermore, transgenic plants overexpressing *FIP1* exhibit delayed growth and flowering as compared to wild type (Figure 4). However, when FIP1 was overexpressed in *fin219* mutant, it turned out that the hyposensitive phenotype was even enhanced, suggesting that FIP1 expression is dependent on the presence of the functional FIN219 to mediate light signal transduction. To confirm the longer hypocotyl phenotype was indeed caused by the overexpression of FIP1, a Northern blot assay was employed to investigate the expression of *FIP1* mRNA in transgenic plants. The result indicated that the transgenic plants containing the 35S::pRTL2-GUS:: FIP1 construct did show highly expressed FIP1 mRNA(figure 5A & 5B), which confirmed that *FIP1* gene overexpression resulted in a less sensitive phenotype with longer hypocotyls in cFR.

FIP1 Expression is Regulated by Auxin and Jasmonic acid

Some GST members in tau class are found to be inducible by 2,4-D(Marrs, 1996: Wagner et al., 2002) and also have a function in detoxification of herbicide(Edwards and Dixon, 2000). We tested the possibility of FIP1 is inducible by phytohormones.

The results of Northern blot assay showed that FIP1 mRNA level was increased by the addition of 50 μM jasmonic acid(figure 6A) and the induction of FIP1 expression by JA occurred very earlier at about 30 min after addition of JA in wild type. However, this induction by JA was much reduced in *fin219* mutant (figure 6C), implying that FIP1 response to JA was dependent on FIN219. On the other hand, *FIP1* mRNA was also induced by 10 μM of 2,4-D within 30 min, and then reduced at 4 hr after addition of 2,4-D (figure 6A & 6B). In addition, *FIP1* mRNA was indeed highly induced by 100 μM herbicide safener Fenclorin (figure 6A). Taken together, FIP1 expression was regulated by different hormones and it maybe participate in plant growth and development.

FIP1 Expression was Regulated by Photoreceptors and Different Light Transducers

Since FIP1 overexpression resulted in a hyposensitive phenotype in cFR and it has been demonstrated to interact with FIN219(figure 1), a positive regulator involved in phyA-mediated light signaling, we tested whether various photoreceptors and light signaling components regulate FIP1 expression. The results of Northern blot analyses indicated that *FIP1* mRNA from 4 day-old seedlings grown under cFR was reduced by *phyA*, *phyB* and *cry1* mutants, and also decreased by several components involved in phA-mediated signaling pathway such as *fin219*, *fhy3*, *spa1*, *cop1* and *hy5* mutants (figure 6A). In addition, *FIP1* mRNA from 4 day-old seedlings grown in R light condition was decreased by photoreceptors *phyA*, *phyB* and *phyA/B* mutants, and also downregulated by *fhy1*, and *fin219* mutants. Intriguingly, *FIP1* expression was upregulated by *spa1*mutation under red light condition (figure 7B). Taken together, expression studies implied that FIP1 is in the downstream of HY5 and regulated by multiple photoreceptors.

GUS::FIP1 Fusion Protein was Localized in Both Cytoplasm and Nucleus, and its Subcellular Location was not Changed by Light

To further understand the subcellular location of FIP1 protein, a FIP1 full-length cDNA was fused with a pRTL2-GUS vector to become a translational fusion construct and subjected for subcellular localization study in onion epidermal cells by particle bombarment method. The result indicated that GUS::FIP1 Fusion Protein was localized in both cytoplasms and nuclei (figure 8C) as compared to the controls (Figure 8A & 8B), and its subcellular location was not changed by light.

DISCUSSION

Previous studies have revealed that FIN219 is involved in phyA-mediated light signaling pathway, induced by auxin rapidly, a constitutively cytoplasmic protein and an extragenic suppressor of COP1, which acts as a repressor of photomorphogenesis in darkness (Hsieh et al., 2000). Staswick et al. (2002) reported that JAR is the same locus as FIN219 in *Arabidopsis*, and JAR1 is an adenylate-forming enzyme that mainly targets jasmonic acid for adenylation to regulate the hormone activity. In addition, JAR1 has been documented to be involved in defense pathways (Nandi et al., 2003; Iavicoli et al., 2003). Here, we reported that a FIN219-interacting protein FIP1 encodes a 217 amino-acid glutathione S-transferase with a predicted molecular weight 24 kD. We also demonstrated by Northern blot analysis that FIP1 was induced by auxin, jasmonic acid and herbicide safener Fenclorin. Besides, FIP1 mRNA expression was affected by multiple photoreceptors and light signaling components, suggesting that FIP1 may play an important role in the integration of light signaling, auxin and defense pathways.

The well characterized aspect of plant GSTs functions was to enhance crops resistance to herbicides and detoxification (Lamoureux and Rusness, 1993). However, plant GSTs exhibit differential expression patterns in response to biotic and abiotic stresses, suggesting that the members of plant GST gene family must play different roles in the interactions between plant growth and environmental cues. Currently, lots of GSTs have been found to be induced by pathogen infection, wounding, heavy metals, plant hormones, reactive oxygen species, heat shock and dehydration etc (Marrs, 1996; Wagner et al., 2002; Smith et al., 2003). In addition, AtGSTZ1 participated in the metabolism of tyrosine (Dixon et al., 2000), and some GSTs were able to associate with auxin and cytokinin to regulate hormone activities, indicating that plant GSTs may play some roles in the metabolic process of plant phytohormones (Zettl et al., 1994; Bilang et al., 1995; Gonneau et al., 1998).

FIP1, AtGSTU20, is a member of the tau class in GST gene family in *Arabidopsis*, in which there are totally 53 genes (Dixon et al., 2002b; Wagner et al., 2002). Phi and tau classes consist of the largest number of members with 28 and 13 genes, respectively. FIP1 expression patterns according to the results of Northern analyses are quite similar to those of FIN219 in several aspects: 1. Both are inducible by auxin and jasmonic acid (figure 6). 2. Both are regulated by light, especially FR. 3. Both seem to be involved in plant defense response. 4. Both are cytoplasmic proteins. Besides, *FIP1* mRNA level was decreased by *fin219* mutation (figure 7) and FIP1 was able to interact with the C-terminal portion of FIN219 (figure 1). Transgenic plants overexpressing the FIP1 gene in wild type background exhibit a hyposensitive

phenotype with longer hypocotyls in cFR (figure 3A), however, this less sensitive response to cFR was even enhanced in *fin219* mutant background (figure 3B), implying that FIP1 function is dependent on the presence of functional FIN219. Taken together, FIP1 and FIN219 may work together to form a complex in response to various signals such as light and phytohormones.

By using different photoreceptors and light signaling mutants, FIP1 mRNA expression was found to be downregulated in *phyA*, *fhy3*, *fin219*, *spa1*, *cop1* and *hy5* mutants under cFR condition. Furthermore, its expression was also reduced in *phyB*, *phyA* mutants and phyAphyB double mutants under cR, suggesting that FIP1 may participate in R light signaling as well in addition to FR involvement and it is working in the downstream of HY5, which is a positive regulator of plant photomorphogenesis. Tepperman et al. (2001) used a DNA microarray approach to examine the gene expression profiles that were induced rapidly by FR irradiation, and found one GST (AAD32887) expression increased promptly, however, the induction was inhibited by *phyA* mutation. In addition, glutathione and a UV-induced GST (AF177944) are involved in a signaling leading to chalcone synthase (Loyall et al., 2000). Recently, genome-wide analysis of gene expression by oligonucleotide microarrays also revealed HY5 involvement in UV-B response of Arabidopsis.Therefore, it seems that a subset of GSTs members are involved in light signaling pathway. It will be interesting to see whether these GST members can interact with each other or have some levels of regulatory relationship to trigger light signaling pathways.

METHODS

Plant materials and growth conditions

Throughout this paper, the wild type is *Arabidopsis thaliana* ecotype Columbia. Blue light photoreceptor mutants *cry1(cry1-304)*, *cry2*(*cry2-1)* and *cry1cry2(cry1-304cry2-1)* double mutants are in the Columbia ecotype as described (Mockler et al., 1999). All other mutants used in this study were described previously (Hsieh et al., 2000; Wang and Deng, 2002).

Surface sterilization and cold treatment of the seeds, and the seedling growth conditions were described previously (Hsieh et al., 2000). Light source used in this study was described by( ) and light intensities used for far-red light, red, blue and white light were , respectively. All the transgenic lines used for phenotypic analysis and Northern blot assays were in T3 homozygous generation.

Yeast two-hybrid assay

A GAL4 yeast two-hybrid system was used for screens of FIN219-interacting partners and protein-protein interaction studies. For pGBT9(+2)-FIN219 construct, a full length of FIN219 cDNA was derived from BamHI digestion of the recombinant plasmid pPZPY122-FIN219, and then ligated into the BamHI site of the BD vector pGBT9(+2) to form a bait construct. An activation domain (AD) fusion of yeast two-hybrid library CD4-10 from *Arabidopsis* Biological Resource Center (ABRC; Ohio State University, Columbus) was used as a prey. AD fusion recombinant plasmids were isolated by a standard method (Sambrook and Russell,2001). The bait fusion plasmid pGBT9 (+2)-FIN219 and the prey recombinant fusion plasmids were then cotransformed into yeast host AH109 (Clontech) and screened for interacting partners on the plates lack of tryptophan, leucine and histidine. The pGBT9 (+2)-FIN219N fusion plasmid was obtained by ligating a BamHI DNA fragment of the recombinant plasmid pPZPY122-FIN219N containing N-terminal 300 amino acids of FIN219, into the BD vector pGBT9(+2). For pGBT9(+2)-FIN219C recombinant plasmid, a BamHI DNA fragment containing the C-terminal 274 amino acids of FIN219 that was amplified by PCR using the primer 219CR-5'-TACGGATCCTACGGGATCATGACTGGCT-3' and the primer 219CL-5'-TACGGATCCTGAGTCAAAACGCTGTGCT-3' (underline indicates the built-in BamHI site) was cloned into the BamHI site of pGBT9(+2). COP1 and HY5 cDNAs were obtained by PCR amplification using the following primers: COP1-L: 5'-TGAGGATCCATGGAAGAGATTTCGACGG-3', COP1-R: 5'-GCAGGATCCTTCCAAATGATGAACCCTACTT-3'. (underline indicates the built-in BamHI site); HY5-L: 5'-CAGCTCGAGATGCAGGAATGGCAAGCGACTAGC-3', HY5-R: 5'-ATACTCGAGATCAAAGGCTTGCATCAGCA-3'(underline indicates the built-in XhoI site). COP1 and HY5 cDNA fragments were cloned into the BamHI and XhoI sites of the BD vector pGBT9(+2), respectively and used for positive control in protein-protein interaction studies. All the above recombinant plasmids were subjected for sequencing to confirm their correctness and in- frame reading.

The putative positive clones from yeast two-hybrid screening were further confirmed by yeast mating method (Ausubel et al., 1999) and colony-lift filter assay(yeast protocols handbook, clontech)..

Recombinant plasmids for plant transformation

To overexpress FIP1 in the *Arabidopsis* Columbia and the *fin219* mutant, a NcoI-BglII DNA fragment containing the full length FIP1 cDNA was obtained by PCR amplification using the following primer pairs:

FIP1-L:5’-TTGCCATGGTTATGGCGAACCTACCGAT-3’(Underline indicates the built-in NcoI site) and FIP1-R: 5’-CATAGATCTCAGAACACATTGGCTTAGCAACA-3’(Underline indicates the built-in BglII site) and ligated into the binary transformation vector pCAMBIA1390-cMyc or pPZP221 with pRTL2-GUS expression cassette. The resulting constructs pCAMBIA1390-cMyc::FIP1 and pPZP221-GUS::FIP1were introduced into the *Agrobacterium* GV3101 by standard method(Sambrook and Russell, 2001) and then transformed into the *Arabidopsis* Columbia and the *fin219* mutant by floral dipping(Clough and Bent, 1998). Transgenic plants containing transgenes were selected with 25 μ g/ml hygromycin for the c-Myc tagged construct and 100. μ g/ml gentalmycin for the GUS tagged construct.

A total of ~40 independent T1 transgenic plants were selected and grown to T2 generation for each transformation construct. Phenotypic analysis was conducted with single T-DNA insertion lines, which were determined by drug resistance test. All transgenic phenotypes reported here were observed in at least 10 independent lines.

RNA gel blot analysis

Total RNA was isolated from 4-day-old seedlings grown in different light conditions using the method described previously (Hsieh et al., 1996). Hormones-induced gene expression studies were conducted according to the method described by Hsieh et al. (2000). Twenty micrograms of total RNA was loaded onto the gel and blotted to the Zeta Probe nynon membrane. A full length of FIP1 cDNA was prepared by PCR for Dig-labelling as a probe according to the manufacture’s procedure (Roche). Furthermore, a pair of gene-specific primers at 3’UTR of *FIP1* was also used to investigate the specificity of FIP1 expression. Hybridization and washing conditions were done by a standard method (Sambrook and Russell, 2001).

Transient assay of subcellular localization by particle bombardment

To construct the GUS-FIP1 fusion protein, a BglII DNA fragment was released from the recombinant plasmid pRSET-B-FIP1 and then cloned into pRTL2-GUS/Nia. The vectors pRTL2-GUS and pRTL2-GUS/Nia alone were also used as control for particle bombardment experiment that was conducted according to the procedures as described (von Arnim and Deng, 1994). After bombardment, the onion epidermal cells were kept in darkess at 25^{o}C overnight, and then some transferred to white light condition for one day, the rest of them still kept in darkness for one more day. GUS staining was performed as described (von Arnim and Deng, 1994).

Pull down assay

To construct the recombinant plasmid pCAL-n::FIN219, a BamHI DNA fragment of

the full length FIN219 was released directly from pPZPY::FIN219 and cloned into the expression vector pCAL-n (Stratagene). For the recombinant pRSET-B::FIP1, a BglII DNA fragment of the full length FIP1 was amplified by PCR using the primer FIP1-L: 5'-TAGAGATCTATGGCGAACCTACCGAT-3' and the primer FIP1-R: 5'-CATAGATCTCAGAACACATTGGCTTAGCAACA-3'. Both recombinant plasmids above were transformed into *E. coli* DH5α and then induced by 1mM IPTG for expression in expression hosts BL21(DE3)Codon plus and BL21(DE3)pLysS, respectively. Recombinant fusion proteins were purified by electroelusion according to manufacture's procedure (BioRad). 5 mg of purified both recombinant proteins were mixed with 50 μl Ni-NTA agarose, made up to 1ml with PBS(1×PBS:140 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, pH 7.3) and then incubated at 4°C with rotation at the speed 30-40 rpm for more than 2 hour. After centrifugation (4000g for 10 sec.), the pellet was washed with washing buffer (50mM Na_2HPO_4, 300mM NaCl, 20mM imidazole, pH8.0) and did centrifugation again. Repeat washing step four times and then eluted by addition of 100 μl elution buffer (50mM Na_2HPO_4, 300mM NaCl, 250mM imidazole, pH8.0). The eluted products were subjected for SDS-PAGE and Western blot analysis by standard methods (Sambrook and Russelle, 2001).

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Figure legends

Figure 1. FIP1 is interacting with the C-terminal region of FIN219.
A. Three different versions of FIN219 and FIP1used for interaction studies. FIN219 full length, FIN219N300 and FIN219C274 are shown with the locations of the coiled-coil (Coil) and GAF domains B. *In vivo* interaction analysis of FIP1 and FIN219 by the yeast GAL4 two-hybrid system. FIN219 proteins were expressed as a bait fusion protein and FIP1 protein as activator domain fusion; transformants were selected on SD media lacking tryptophan, leucine and histidine: trp-leu-his. **Figure 2. A diagram of *FIP1* gene and alignment of its amino acid sequence with a GST member At1g78340.**
A. The FIP1 gene in Arabidopsis is composed of two exons and one intron, and encodes a 217 amino- acid protein. B. Alignment of FIP1 with the closest GST member At1g78340 with 67% identity at amino acid level.

Figure 3. Phenotypes of transgenic plants overexpressing FIP1 gene in *Arabidopsis* Columbia and *fin219* mutant.
A. The phenotypes of *FIP1* transgenic plant overexpression *FIP1* under far red light (FR) irradiation. Col.(left) was used as a wild-type control. *fin219*(right) mutant (right) is antother control. T2 transgenic seedlings overexpressing the *FIP1* display a hyposensitive phenotype under continuous FR irradiation for three days (the middle). B. Transgenic plants overexpressing FIP1 are dependent on functional FIN219. The transgenic line GusFIP1/*219* #4-12 expressed in *fin219* mutant was shown in the middle. Col.(left) was used as a wild-type control. *fin219* mutant (right) is antother control.

Figure 4. Phenotypes at adult stage of transgenic plants overexpressing FIP1 gene in *Arabidopsis* Columbia.
Transgenic plants containing the FIP1 overexpressing construct exhibit a delay flowering and dwarf phenotype at Day 19 (A) and Day 28 (B) in white light condition.

Figure 5. Northern blot analysis of transgenic plants expressing the *FIP1* gene in *Arabidopsis* Columbia.
Investigate FIP1 gene expression in 3-day-old seedlings of transgenic lines #412 and #963 grown in continous far-red light. 10 μ g of total RNA was loaded onto the gel, carried out RNA electrophoresis and then blotted to membranes. The membrane was hybridized with a dig-labelled FIP1 full-length cDNA. The upper arrow in (B) indicated transgene signal and the bottom one is endogenous *FIP1* signal.

Figure 6. Northern blot analyses of *FIP1* gene expression by different hormones and herbicide safeners.

FIP1 mRNA induction were examined by the addition of different hormones indicated above and a herbicide safener Fenclorin (A) and by a time course of auxin 2,4-D addition (B). 6-day-old seedlings grown in light were transferred to a liquid medium in the presence or absence of various hormones and safeners. Seedlings were harvested 3 hours later or different time period as indicated above.

C. FIP1 induction by JA is more sensitive in Col than that in *fin219* mutant. 6-day-old seedlings grown in light were transferred to a liquid medium in the presence or absence of 50μM jasmonic acid. Seedlings were harvested in different time course as indicated above

Figure 7. Northern blot analyses of *FIP1* gene expression in photoreceptor mutants and various light signaling mutants under continuous FR (cFR) and red light conditions.

A. *FIP1* expression in different mutants under far red light. Equal amount of 20 μg total RNA isolated from 4-day-old seedlings of wild-type Columbia and different mutants grown in cFR was used each lane. B. *FIP1* expression in different mutants under continuous red light (cR). Equal amount of 15 μg total RNA was used each lane.

Figure 8. Transient assay of subcellular localization of GUS::FIP1 fusion protein in onion epidermal cells by particle bombardment.

A. The construct containing GUS only is distributed evenly in both cytosol and nucleus as shown in a GUS staining of onion epidermal cells. B. The construct containing GUS-Nia (nuclear localization signal) is only restricted in the nucleus as shown in a GUS staining of onion epidermal cells. C. GUS::FIP1 fusion proteins are located in the cytosol and the nucleus as shown in a GUS staining of onion epidermal cells.

Figure 1

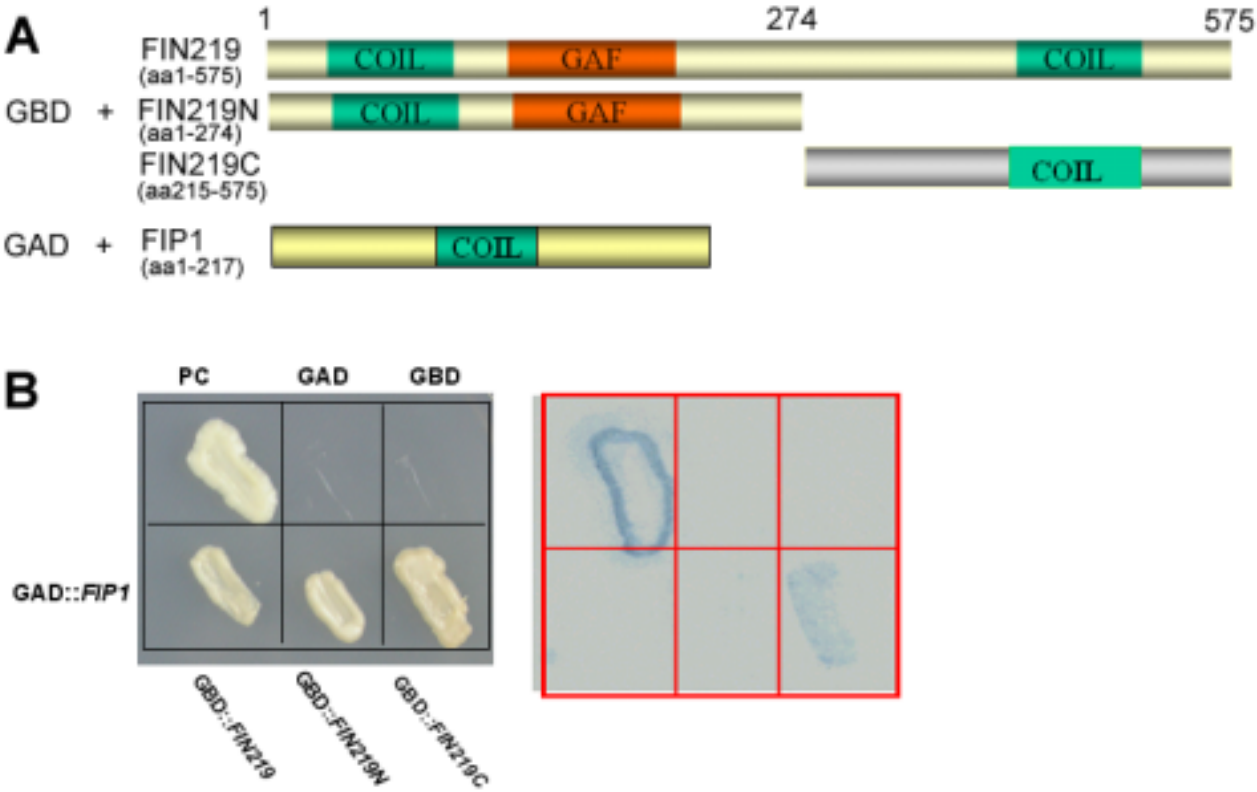
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Figure 2

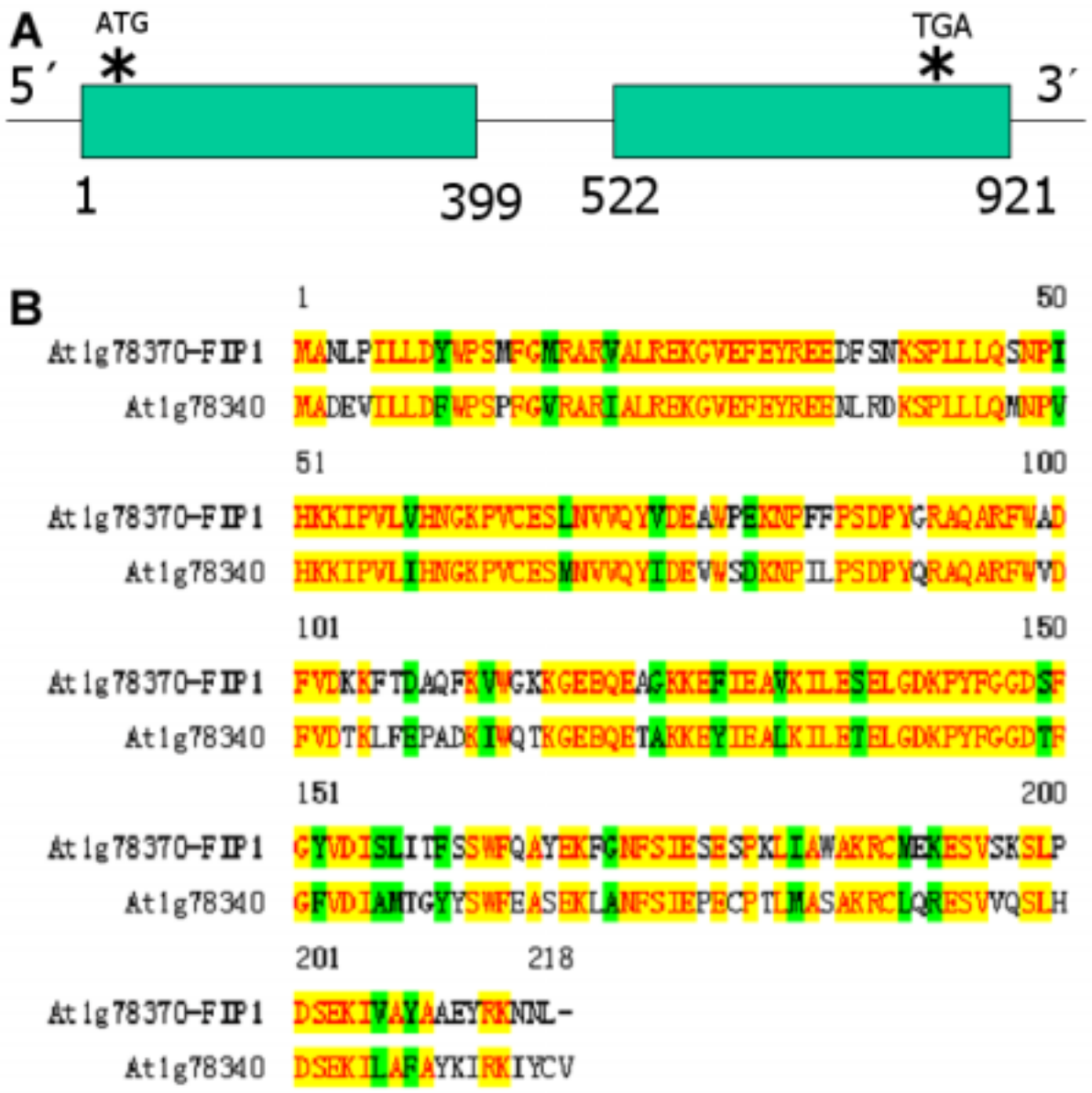
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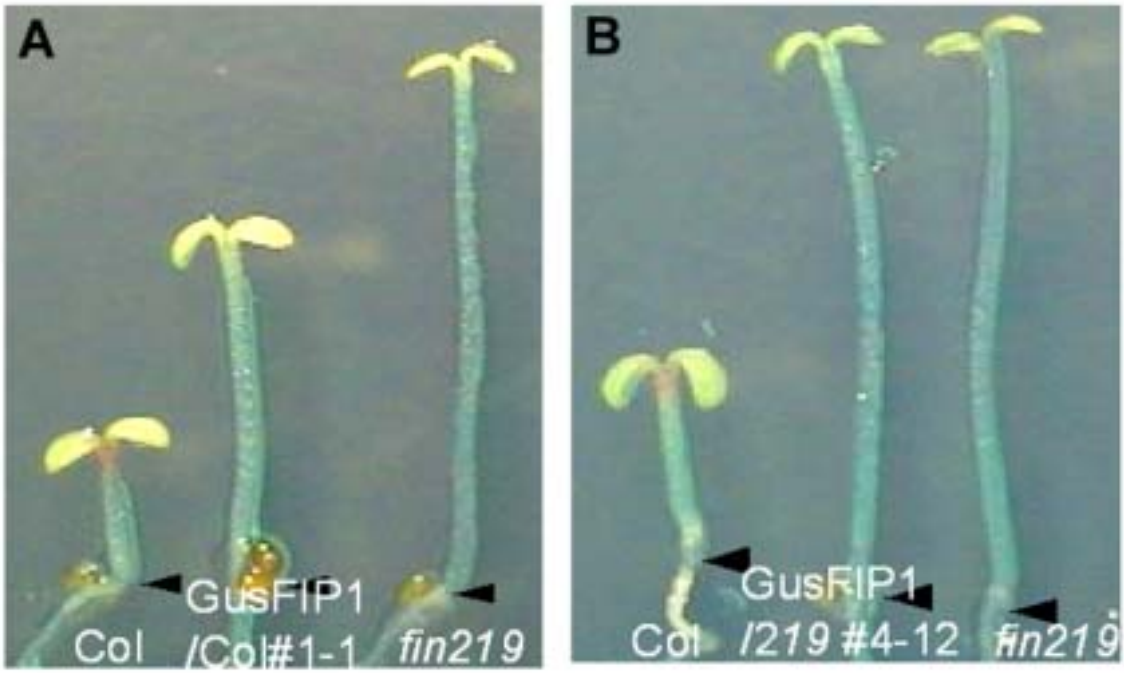
Figure 3



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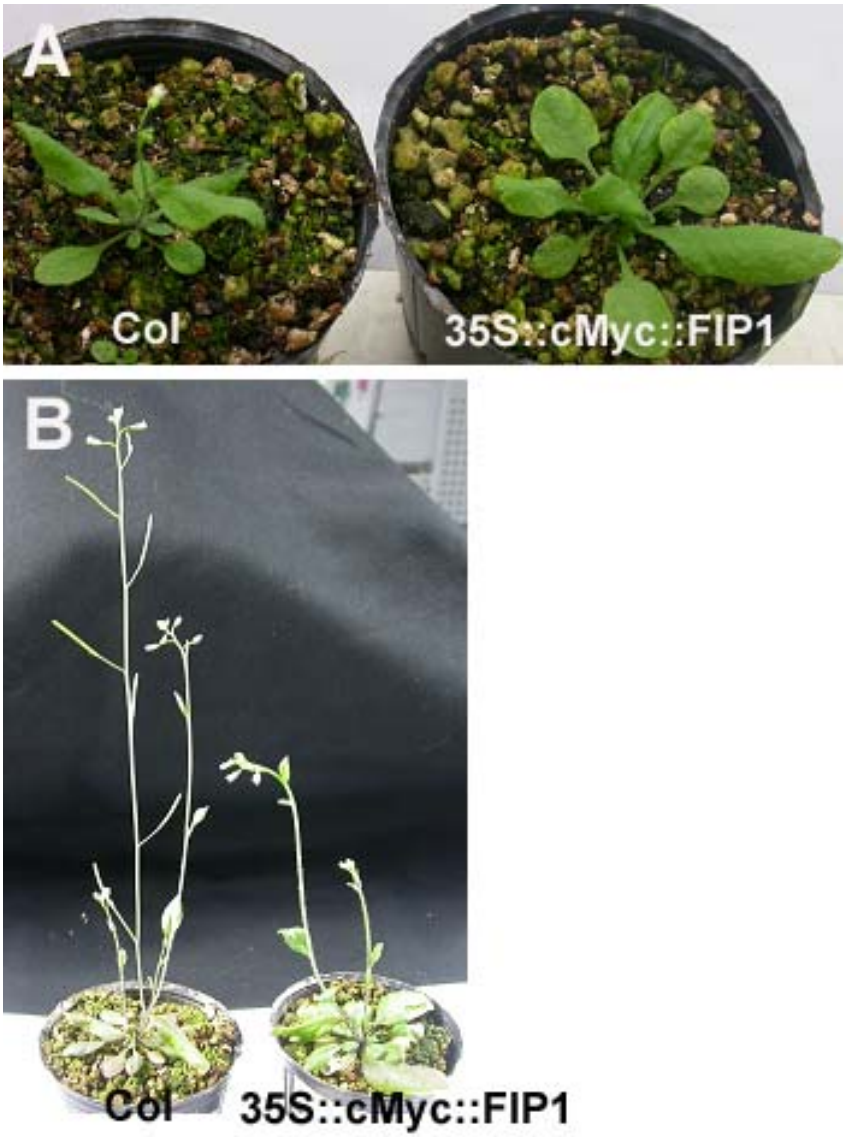

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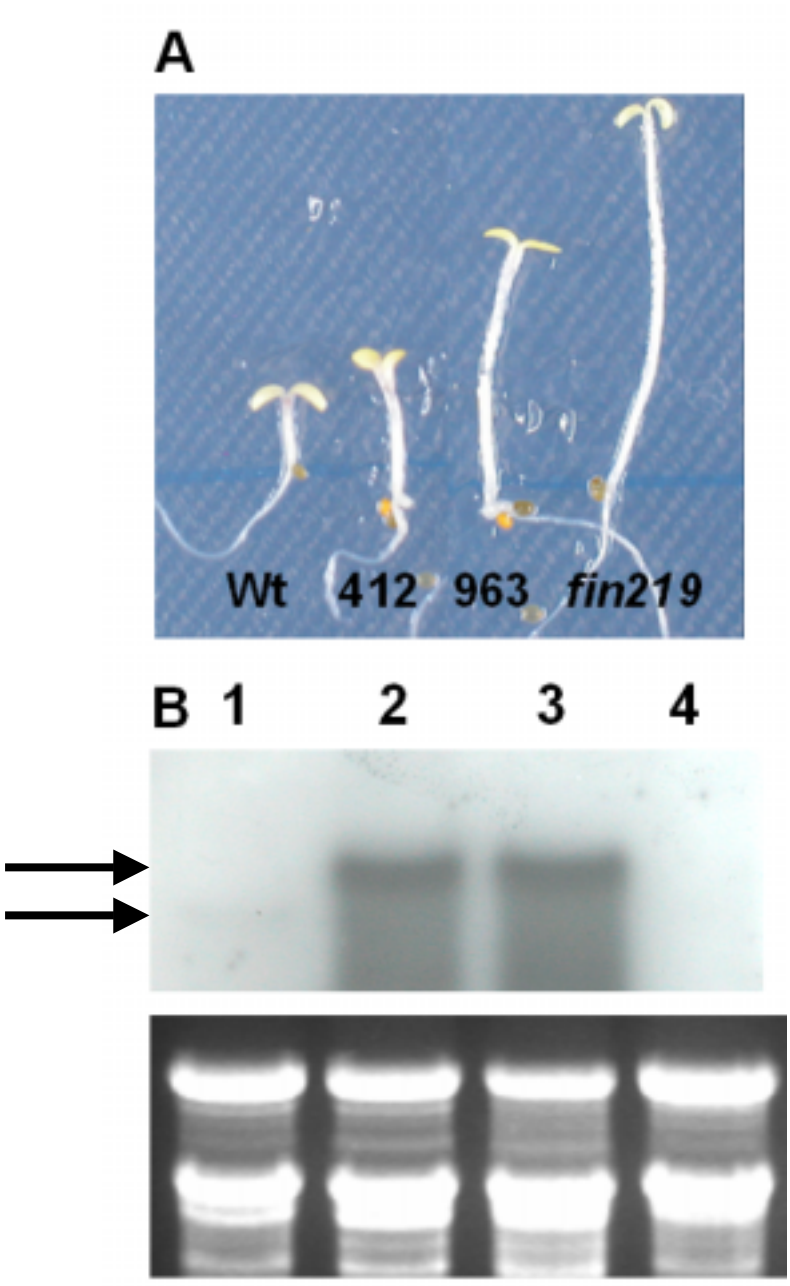
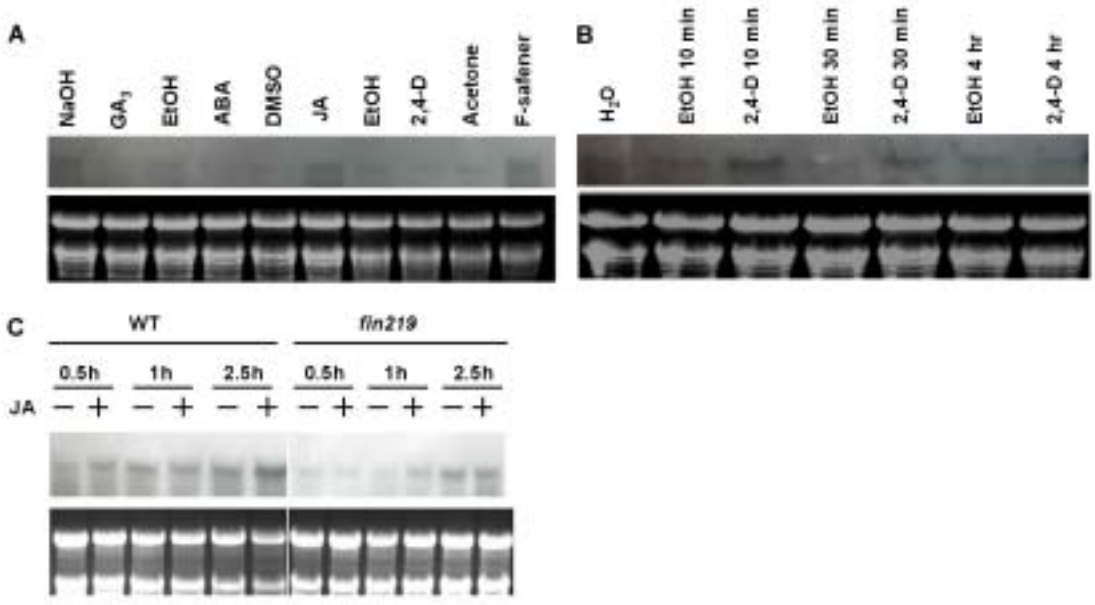


Figure 6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Acetone
F-safener
B
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2,4-D 10 min
EtOH 30 min
2,4-D 30 min
EtOH 4 hr
2,4-D 4 hr
C
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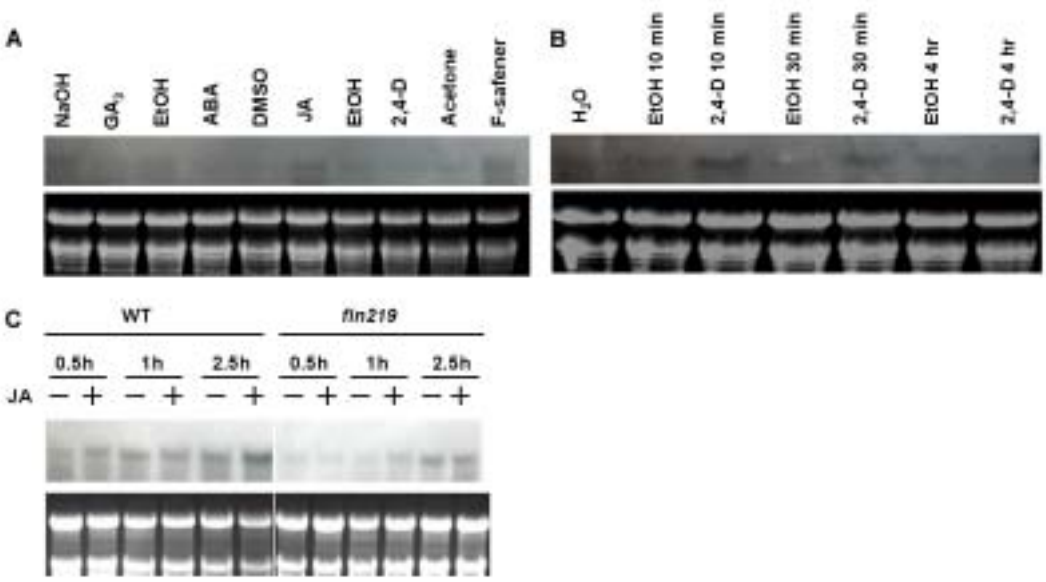
Figure 7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2,4-D 10 min
EtOH 30 min
2,4-D 30 min
EtOH 4 hr
2,4-D 4 hr
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Figure 8