

Spaceflight studies identify a gene encoding an intermediate filament involved in tropism pathways

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Highlights:

- Experiments to study tropisms were performed with plants on the ISS. Seedlings grown in space were frozen and returned for analysis via RNASeq.
- Five genes showing high differences in expression, pertaining to light and/or gravity perception categories, were selected.
- Mutants of these 5 genes were studied with tropism assays on the ground.
- One of these genes encoded an intermediate filament (neurofilament light protein).
- *NFL* appears to play a role in phototropism and gravitropism pathways.

Keywords:

Cytoskeleton, gravitropism, intermediate filaments, microgravity, neurofilament light protein, phototropism, spaceflight.

Abstract

We performed a series of experiments to study the interaction between phototropism and gravitropism in *Arabidopsis thaliana* as part of the Seedling Growth Project on the International Space Station. Red-light-based and blue-light-based phototropism were examined in microgravity and at 1g, a control that was produced by an on-board centrifuge. At the end of the experiments, seedlings were frozen and brought back to Earth for gene profiling studies via RNASeq methods. In this paper, we focus on five genes identified in these space studies by their differential expression in space: one involved in auxin transport and four others encoding genes for: a methyltransferase subunit, a transmembrane protein, a transcription factor for endodermis formation, and a cytoskeletal element (an intermediate filament protein). Time course studies using mutant strains of these five genes were performed for blue-light and red-light phototropism studies as well as for gravitropism assays on ground. Interestingly, all five of the genes had some effects on all the tropisms under the conditions studied. RT-PCR analyses examined expression of the five genes in wild-type seedlings during blue-light-based phototropism. Previous studies have supported a role of both microfilaments and microtubules in tropism pathways and, specifically, in gravity sensing. However, the most interesting finding of the present space studies is that *NFL*, a gene encoding an intermediate filament protein, plays a role in phototropism and gravitropism, which opens the possibility that this cytoskeletal element modulates signal transduction in plants.

1.Introduction

Plants have evolved sensitive mechanisms to perceive and respond to their environment (Vandenbrink et al., 2014; Legris et al., 2017). Light and gravity are among the most critical stimuli in early plant development (Goyal et al., 2013; Vandenbrink and Kiss, 2019). Light provides the energy for photosynthesis, important directional information (e.g., via phototropins), and signals for development (e.g., via phytochromes). Throughout their life cycle, plants use gravity to orient and coordinate their growth to maximize access to light, water, and nutrients. Plants also continuously integrate all of the information received by their sensory systems to adjust their growth to their present environmental conditions.

Phototropism is the directed growth of a plant relative to the direction of a light stimulus (Christie, 2007; Holm et al., 2013). In flowering plants, phototropism typically is induced by blue light. Stems and shoots are generally positively phototropic, growing toward the light, and roots usually exhibit negative phototropism in response to unidirectional blue light (Correll and Kiss, 2002). However, in spaceflight studies, we also have identified a novel positive phototropism in response to red light in both roots and shoots (Kiss et al., 2012; Vandenbrink et al., 2016). Gravitropism is directed growth in response to the gravity vector, and, in general, stems and shoots grow up away from gravity while roots grow downwards toward the gravity vector (Kiss, 2000; Molas and Kiss, 2009; Morita, 2010).

In order to gain insights into the mechanisms of phototropism and gravitropism, we performed a series of spaceflight experiments (Vandenbrink and Kiss, 2016; Vandenbrink et al., 2016), termed Seedling Growth (SG), on the International Space Station (ISS). Images captured from the ISS experiments in orbit were used to analyze growth, development, and curvature of plants in varying light and gravity conditions (by using an on-board centrifuge). At the end of the

experiments, the seedlings were frozen and returned to Earth for gene expression studies (Valbuena et al., 2018; Vandenbrink et al., 2019, Herranz et al., 2019).

Analysis of data produced from this suite of experiments revealed novel tropistic responses in a continuum of gravity conditions (Vandenbrink et al., 2016). In addition, results of the RNAseq data from our SG spaceflight studies show that multiple genes and molecular pathways are affected by microgravity (Vandenbrink et al., 2019; Villacampa et al., 2021). After extensive studies, we were particularly interested in several genes (Table 1) that show alterations in tropisms based on our physiological characterization. These genes were selected by determining which characterized genes showed the greatest differential transcription when comparing seedlings grown in microgravity to the 1-g control (Vandenbrink et al., 2019).

In this paper, we focus on plants that are mutated in five genes based on the above analysis resulting from our spaceflight experiments. We also confirm selected results by using RT-PCR analysis to study the five selected genes and suggest that a gene for an intermediate filament (*NFL*; neurofilament light protein) is involved in tropism pathways in plants. The observation that intermediate filaments may have a role in tropism pathways adds to the extensive database that the cytoskeleton plays a significant role in the mechanisms of gravitropism and phototropism (Molas and Kiss, 2009; Morita, 2010; Blancaflor, 2013; Nakamura et al., 2019).

2. Materials and Methods

2.1 Spaceflight experiments

Experiments with seeds of *Arabidopsis thaliana* ecotype *Landsberg erecta* (Ler) were performed on the International Space Station (ISS), and the details are provided in Vandenbrink et al.

(2019). Briefly, space experiments (termed the Seedling Growth project) were conducted utilizing the European Modular Cultivation System (EMCS) which had two centrifuges and environmental controls (Brinckmann, 2005; Kiss et al., 2014). The experiments were initiated via hydration of the seeds, and seedlings were grown in microgravity and in a 1.0-g control as shown in the detailed timeline provided in Vandenbrink et al. (2019).

At the end of the experiments, seedlings were frozen and stored at -80°C, returned to Earth, and finally preserved with RNAlater® (ThermoFisher Scientific cat # AM7021) for subsequent RNAseq analysis. Raw data from these analyses were deposited into NASA's GeneLab under accession number GLDS 251 (<https://genelab.nasa.gov/>).

2.2. Seed source for ground experiments

A. thaliana seeds for the five single gene knockout mutants (Table 1) were received from the Arabidopsis Biological Resource Center (<https://www.arabidopsis.org/>). For each mutant homozygous genotype, several plants were propagated, and seeds harvested. Wild type seeds, Columbia (WT), originating from the same source were propagated in the lab.

2.3. Seedling culturing in ground experiments

Prior the experiments, seeds were surface sterilized with 70% (v/v) ethanol containing a drop of Triton X-100 (200 ml) for 7 min, rinsed twice with 95% (v/v) ethanol for 1 min each, and then rinsed twice with sterilized water. Surface sterilized seeds were plated on 1.2% (w/v) agar with half-strength Murashige and Skoog salts medium containing also 1% (w/v) sucrose at pH=5.5 (Kiss et al., 1997). When solidified, agar in the gridded square Petri dishes 100x100x15 mm (Cat #60872, VWR International, LLC) was covered with a piece of sterilized nitrocellulose film

(Cat# V7131, Promega Corp., Madison, WI, USA). Twelve seeds were placed in two rows into each dish. Plates were wrapped with Parafilm and left in refrigerator for 72 h to promote uniform germination. Then for the light-grown group, plates were placed vertically under a continuous white fluorescent light at $120\text{--}160\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$ from above for 96 h at room temperature. For the dark-grown group, plates were kept under light for only 24 h to trigger germination and then moved to the dark for 96 h.

2.4. Gravitropism and phototropism experiments on the ground

These experiments were performed at room temperature. Each experiment was repeated three times with 2-3 plates for each genotype. For the gravitropism assays, plates were reoriented 90° in the dark, and a series of images were taken at 0, 0.5, 2, 4, 8, 24, 48, and 72 h under a dim short-term green light with Canon EDS Rebel T6 digital camera. For the blue or red light phototropism studies, plates were illuminated with the unidirectional blue $60\text{--}65\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$ or red $35\text{--}40\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$ light from one side. For the blue light experiments, seedling images were taken at 0, 0.5, 1, 2, 4, 8, 24, and 48 h after the experiment start. For the red light experiments, images were taken 24 h after the experiment start. From the images, seedling shoot and root length and also shoot and root curvatures were analyzed with Fiji software <https://imagej.net/Fiji>.

2.5. RNA extraction and analyses in ground experiments

To study changes in gene expression during the blue light treatment, we grew wild-type seedlings similarly as mutant seedlings for the phototropism experiments. Seedlings were harvested at the blue light treatment start and after 8, 24, and 48 h. To amass enough biomass for gene expression analysis, each shoot or root sample was combined from 10-14 seedlings and

placed into 2 ml microcentrifuge tube with one 4.5 mm steel ball inside (Daisy BBs, Daisy Outdoor Products, Rogers, AR, USA) for tissue grinding. Three replicates for shoot and root samples were harvested for each genotype at each time point. Total RNA was extracted with TRIzol reagent (Cat # 15596018, Thermo Fisher Scientific) based on the manufacturer instructions.

Plant tissues were homogenized with TissueLyser LT machine (Qiagen). RNA samples were diluted to 5 µg/µl and used for cDNA synthesis with High capacity cDNA Reverse Transcription Kit (Cat # 4368814, Thermo Fisher Scientific). Obtained cDNA was 1: 10 diluted and used for qPCR tests with gene specific primers (Supplementary table S1). *ACT8* gene expression was used as internal control via primers described in Morita et al. (2006). While there are limitations to any reference gene, *ACT8* is one of the most widely used reference genes in gene expression studies. In Remans et al. (2008), the authors explored 13 potential reference genes for *Arabidopsis thaliana*, and actin was number four by stability. In more recent studies, *ACT8* also has been shown to be a good reference gene (Joseph et al., 2018). Other primers were designed with Primer Blast at <https://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi>.

For quantitative RT-PCR analyses, we used a QuantStudio 6 (Applied Biosystems) machine with 384-well plates and 12 µl reactions with PowerUp SYBR Green Master Mix (Cat #A25742, Applied Biosystems). Procedures were carried out as follows: 95°C for 10 min and 40 cycles of 95°C for 15 s and 60°C for 35 s, and 95°C for 15 s, 60°C for 15 s, 95°C for 15 s and followed with the melting curve cycle.

2.6. Statistical analyses

Data from the gravitropism and phototropism experiments were not normal, so nonparametric Wilcoxon rank sum tests were used to compare each mutant genotype with the wild-type seedlings. RNA data were analyzed with the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001) so that at each time point shows fold in a gene expression compared to our reference gene, *ACT8*. We calculated $\Delta\Delta Ct = (Ct_{Target} - Ct_{ACT8})_{Time\ x} - (Ct_{Target} - Ct_{ACT8})_{Time\ 0}$. $\Delta\Delta Ct$ values for every gene were compared across the experimental time points with ANOVA II tests with time, tissue and their interaction as factors and also for shoot and root samples separately with ANOVA I tests with time as factor. All analyses were performed with the RGui 64 bit R 4.0.2. for Windows with Rcmdr package (R Core Team 2020).

3. Results

3.1 RNA-seq analyses of *Arabidopsis* seedlings from the spaceflight experiment

Analysis of differential gene expression of *Arabidopsis* seedlings grown in microgravity vs. a 1-g control was conducted with DESeq. We were particularly interested in the genes characterized as differentially expressed in pathways pertaining to light perception or the biogenesis of light perception machinery. From the list of genes identified via DESeq as well as GAGE (Generally Applicable Gene-set Enrichment) pathway analysis, we have selected four genes to confirm as playing a role in the interaction of light and gravity responses (mutants of these genes are shown in Table 1).

In addition, a fifth gene (*TMP*) that was differentially expressed was selected that did not map to any known KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways; Table 1). This gene belongs to the PIN family of genes which has previously been characterized as playing a role in

the perception and response to gravity via auxin their role in auxin transport (Haga and Sakai, 2012; Baster et al., 2013; Löffke et al., 2013).

In addition, the five genes selected showed very large differential transcriptional effects when comparing seedlings grown in microgravity conditions versus the 1-g control. Genes that were uncharacterized in the databases at the time were not considered.

3.2 Gravitropism experiments

We studied gravitropism in these mutants (Table 1) which were identified based on our previous spaceflight studies (Vandenbrink et al., 2019). In reorientation studies, shoot curvatures were reduced in several mutant genotypes compared to the wild-type seedlings (Fig. 1A and B). This effect was similar in *sgr*, *abc*, and *nfl* mutants in both light-grown and dark-grown conditions. However, this reduction was observed only in light-grown *mts* seedlings. For light-grown *sgr* seedlings, shoot curvatures were already reduced at 0.5 h after reorientation. Other mutant seedlings had significant reductions only after 24 h or later. Light-grown *nfl* seedlings had the strongest difference compared to the wild-type at 24 – 72 h after reorientation.

Root curvatures were reduced at 0.5 – 8 h after reorientation in dark-grown *tmp* and *mts* seedlings (Fig. 1 C and D). However, at 72 h after reorientation, root curvatures were increased in *sgr*, *tmp*, and *abc* light-grown seedlings and remained reduced in *tmp* dark-grown seedlings compared to the corresponding wild-type.

In some mutant genotypes, shoot growth in seedlings was differently affected by reorientation in dark-grown and light-grown seedlings (Table 2, Supplementary table S2). Compared to the corresponding wild-type, shoot growth was reduced only in dark-grown *mts* seedlings at 24 – 72 h. Shoot growth was increased only in light-grown *tmp* seedlings at 48 – 72 h. Interestingly, in *sgr*, *nfl*, and *abc* genotypes, shoot growth was increased in light-grown but reduced in dark-

grown seedlings at 72 h. In addition, increased shoot growth observed at 0.5 h after reorientation in *mts*, *sgr*, *nfl*, and *abc* genotypes switched to reduced growth at later times 24-72 h.

In *sgr*, *nfl*, and *abc* genotypes root growth was reduced only at 24 h or less after reorientation (Table 2, Supplementary table S2).

3.3 Blue-light-based phototropism

We also studied blue-light-based phototropism in the ground experiments with the mutants identified in the spaceflight project. Interestingly, 1-8 h after unilateral blue, shoot curvatures were increased in all mutant genotypes from light-grown seedlings but reduced at 4-8 h in dark-grown seedling when compared to the corresponding wild-type (Fig. 2A and B). After 48 h of blue illumination, in light-grown seedlings, only *mts* mutants still had increased shoot curvatures, while all dark-grown genotypes except *mts* remained significantly reduced.

Root curvatures were different from the wild type only in light-grown but not dark-grown seedlings (Fig. 2C and D). Root curvatures in light-grown *mts* and *abc* genotypes were increased from 8 to 48 h of the experiment, in *nfl* and *sgr* genotypes from 8 to 24 h, and in *tmp* only at 8 h. Images of representative seedlings from which we obtained the above time course data are shown in Figure 3. For example, in terms of gravitropism, at the 48 h point in a time course study, shoot curvature of the *nfl* mutant seedlings grown in the light is reduced and delayed relative to the WT, while roots remain unaffected (Fig. 3). The reduction in shoot curvature in the blue-light-based phototropism studies of the *nfl* mutant (relative to the WT) also is apparent from the images of the light-grown seedlings (Fig. 3).

The shoot growth rates in all mutants differ from the wild-type seedlings at least at some time points (Table 3, Supplementary Table S3). However, the effects of the blue light treatment for

the light-grown and dark-grown seedling were different. In general, light-grown *sgr* and *nfl* seedlings had increased shoot growth while dark-grown seedlings had reduced shoot growth compared to the corresponding wild type. For *tmp* and *abc* mutants, shoot growth was initially slower than in the wild type in light-grown seedlings, but after 24 h, shoot growth was faster than in the wild-type seedlings. In contrast, in dark-grown *tmp* and *abc* seedlings, shoot growth was reduced at 0.5 – 48 or 36 - 48 h, respectively, compared to the wild-type. Only light-grown *mts* seedlings had increased shoot growth at the end of the experiment (36 – 48 h), while dark-grown seedlings had no difference when compared to the wild-type.

The root growth rate was initially decreased (0.5 h) in light-grown *tmp* and *sgr* mutants when compared to the corresponding wild-type (Table 3, Supplementary Table S3). However, later in the time course studies, this effect disappeared in *tmp* seedlings and, in fact, increased at 8 – 48 h in *sgr* seedlings. At mid to late stages of the unilateral blue-light time course studies, root growth was also increased in *nfl*, *mts*, and *abc* light-grown mutants. In *tmp* dark-grown mutants, root growth was slower than in the wild-type seedlings.

3.4 Red-light-based phototropism

While blue-light phototropism is robust on the ground, red-light-based phototropism is difficult to detect in ground-based studies (Kiss et al., 2003) although it is readily apparent in microgravity conditions during spaceflight (Vandenbrink et al., 2016). Nevertheless, in our ground experiments, while median shoot and root curvatures in light-grown wild-type seedlings were slightly negative relative to red light, surprisingly, several mutant genotypes had shoot and root curvature toward the red light (Fig. 4). After 24 h red light treatment, in light-grown *nfl* mutants, shoot and root curvatures were increased compared with the wild-type (Fig. 4A and C).

In light-grown *tmp* seedlings, shoot curvature was increased (Fig. 4A), and in *mts* and *abc* seedlings, root curvatures were increased (Fig. 4C). In dark-grown wild-type and mutant genotypes, shoot curvature was positive in magnitude, but no significant differences were found. In dark-grown seedlings, all genotypes had negative root curvature. In general, dark-grown seedlings had broader variation in their shoot and root curvature responses compared to light-grown seedlings (Fig. 4D).

After 24 h of red light treatment, shoot growth was reduced in several mutant genotypes: dark-grown *tmp*, *nfl*, and *abc* and light-grown *sgr* (Table 4, Supplementary Table S4). Root growth was increased in dark-grown *mts* and *sgr*. In *nfl* mutants, light-grown seedlings had reduced root growth, while dark-grown seedlings had increased root growth when compared to the corresponding wild-type (Table 4).

RNA analyses from WT wild-type seedlings during time course studies of blue-light-based phototropism

As indicated in Figure 5 for each gene tested by RT-PCR studies, ANOVA II comparisons showed significant effects of time and tissue for *NFL* (time $F=5.601$, $P=0.008$; tissue $F=9.854$, $P=0.006$) and *ABC* (time $F=4.550$, $P=0.019$; tissue $F=6.854$, $P=0.019$) genes and also significant effect of time for *MTS* gene ($F=7.395$, $P=0.003$). No significant effects were found for *SGR* and *TMP* genes. For shoots, ANOVA I tests showed significant effect of time for *MTS* gene ($F=16.22$, $P=0.0009$). For roots, ANOVA I tests showed significant effects of time for *NFL* gene ($F=4.669$, $P=0.036$) and marginally significant for *ABC* gene ($F=3.46$, $P=0.079$).

4. Discussion

4.1 Mutants identified in spaceflight show that a set of genes have a newly demonstrated effect on phototropism and gravitropism

We performed a series of experiments with *Arabidopsis* seedlings (grown at various gravity levels from microgravity to 1-g) on the International Space Station (ISS) as part of the joint NASA/ESA project termed Seedling Growth (Vandenbrink and Kiss, 2016; Herranz et al., 2019; Manzano et al., 2020; Vandenbrink et al., 2019; Villacampa et al., 2021). From the list of genes showing differential expression in the seedlings, identified via RNASeq analyses following the spaceflight studies (Vandenbrink et al., 2019), we selected five genes (see Table 1) as excellent candidates to study the role of the interaction of light and gravity responses. Based on these space experiments, we selected these genes by determining which characterized genes showed very large differential transcriptional effect when comparing seedlings grown in microgravity conditions versus the 1-g control, as described in Vandenbrink et al. (2019). The summary of physiological studies (performed in this paper) of the five mutants in these genes is provided in Table 5.

In ground-based studies of these mutant strains, we studied the effects of the mutated genes on blue-light and red-light-based tropisms as well as on gravitropism (Table 5). It is important to note while plants exhibit a robust blue-light-induced phototropism in ground studies, the red-light-based response is weak and difficult to detect on the ground (Fig. 4; see also Kiss et al., 2003) while it is readily apparent in microgravity in spacecraft in low Earth orbit (Millar et al., 2010).

Interestingly, all five of the genes (*MTS*, *TMP*, *SGR*, *NFL*, and *ABC*) had effects on red and blue phototropism as well as gravitropism under some conditions. While in some cases there was attenuation and in other cases there was stimulation of the tropism (Table 5), there were no instances detected in which there was an opposite effect by the influence of a gene on a tropism in roots and shoots. However, differences in effects were noted when comparing tropisms under light or dark conditions. For instance, *ABC* attenuated shoot curvature in light-grown seedlings during most of the time course during blue light phototropism, but had the opposite effect in dark-grown seedlings (Fig. 2; Table 5). Differential effects of light conditions on mutants and transgenic plants in tropism pathways have been noted by our group (e.g., Hopkins and Kiss, 2012) and others (Park et al., 2018).

4.2 Phototropism studies

The studies that showed the clearest influence on tropisms were the blue-light-based phototropism assays in which all five genes studied has an effect on this tropism in both roots and shoots. In general, all five genes attenuated blue-light phototropism in light-grown seedlings while these genes promoted the tropism in dark-grown seedlings.

These effects were more minimal in roots of dark-grown seedlings probably since root growth is inhibited in these conditions (Correll and Kiss, 2005; Silva-Navas et al., 2015). While there are some relatively minor effects throughout the red-light phototropism curvature studies, it is difficult to distill specific effects as this is a weak phototropic response on the ground (Kiss et al., 2003; Millar et al., 2010). Nevertheless, the one gene that shows an effect in both roots and shoots is *NFL* which attenuates the red-light phototropic response (Fig. 4).

4.3 Gravitropism studies

While our space experiments initially focused on phototropism (Vandenbrink et al., 2019), since we also studied different gravity levels from microgravity to reduced gravity to 1g (Kiss et al., 2012; Vandenbrink et al., 2016), we also considered the effects of these genes on gravitropism assays. Interestingly for *MTS*, and *TMP*, the effects were more prominent in roots, where these three genes promoted gravitropism in light grown. The *sgr* mutant is a good internal control for our studies since *SGR* has been shown to be involved in shoot gravitropism by encoding for a transcription factor involved with endodermis formation (Fukaki et al., 1996; Morita, 2010). As expected, *SGR* promotes gravitropism in both light-grown and dark-grown shoots of plants, but there was no effect on gravitropism in roots where the gene is not expressed.

4.4 Growth studies

In addition to experiments on the effects of the five genes on tropisms, we also studied the effects on growth in roots and shoots of seedlings. In most cases, the changes in growth rates did not account for the difference in tropistic response between the mutant and its parental WT. This was especially significant for the *NFL* gene. For instance, in gravitropism studies of shoots in light-grown seedlings, at 72 h in the time course (Fig. 1), gravitropism in the *nfl* mutant was inhibited (77% of the WT) while the growth rate was not significantly different (Table 2). In fact, in another case at 48 h in the time course of gravitropism in light-grown seedlings (Fig. 1), the *abc* mutant was inhibited (80% of the WT) while the growth rate of this mutant was significantly greater than the WT at this point in the time course studies (Table 2).

Similar observations concerning the relationship between phototropism and growth were noted, and also affected especially to the *NFL* gene. For example, at 8 h in the time course of blue-light

phototropism in light-grown seedlings (Fig. 2), the *nfl* mutant exhibited an enhanced curvature (146% of the WT), but there was no significant difference in growth relative to the WT (Table 3). The lack of correlation between changes in the tropistic responses in mutants and growth effects have been noted in previous studies from our laboratory (e.g., Kiss et al., 2003) and those of other groups (Muthert et al., 2020).

4.5 RT-PCR studies of genes involved in blue-light-based phototropism

Since blue-light phototropism is robust in both roots and shoots, we studied expression of our five candidate genes with RT-PCR during this tropism in light-grown WT seedlings. For three of the five genes (*MTS*, *NFL*, and *ABC*), there were changes in gene expression during the time course studies of blue phototropism.

For *MTS*, the time course of curvature studies suggest that this gene attenuates phototropism in the shoots, and RT-PCR studies show a corresponding decrease in expression of this gene. *MTS* has been shown to be involved in organ growth and regulation of cell cycle (Balasubramani et al., 2021), but this is the first report of it being involved in tropism pathways.

Like the PIN family, ABC proteins have been shown to play a role in gravitropism and phototropism (Kumar et al., 2011; Okamoto et al., 2016). ABC proteins, ATP-BINDING CASSETTE (ABC) transporters, are one of the largest superfamily of transmembrane transporter proteins that utilize ATP to carry out translocation of substrates across membranes (Borghi et al., 2019). In plants, ABC proteins shuttle a variety of substrates such as lipids, carboxylates, heavy metals, and hormones such as auxin, across a variety of biological membranes (Sharom et al., 2011). Our time course of curvature studies suggest that *ABC* attenuates blue-light phototropism

in roots. However, in contrast, RT-PCR experiments suggest that *ABC* gene expression increases during the same time.

In some ways, the most important finding of our studies is that *NFL* plays a role in gravitropism and phototropism. *NFL* proteins (Nefedova et al., 2017) are particularly fascinating because of their role as intermediate filaments (IF). While the existence of IFs in plants has been debated, new molecular and cytological evidence confirms that IFs do occur in plant cells (Soda et al., 2018; Utsunomiya et al., 2020). The importance of *NFL* also is suggested by data in the Klepikova Atlas (used in the TAIR database; <https://www.arabidopsis.org/>), which provides tissue-specific profiling for many genes throughout the *Arabidopsis thaliana* genome (Klepikova et al., 2016). Thus, tissue-specific profiling indicates that *NFL* shows high expression in the roots of 1-day old seedlings, and moderate expression in aerial tissue. Many studies have shown that the microtubules and the actin cytoskeleton play a major role in gravitropism and phototropism (Kiss, 2000; Molas and Kiss, 2009; Nakamura et al., 2019). Actually, one of the most important open points in plant gravitational biology is the identification of the cellular mechanism of gravity sensing in “non-professional” cells, i.e. those cells lacking specialized gravisensing organelles, such as statoliths (Van Loon, 2009). Recent experiments strongly suggest that the cortical cytoskeleton could be the major cellular mechanosensory apparatus (Roeder et al., 2021) and hence could play the role of sensing gravity changes. The involvement of a cytoskeletal protein in the cellular response to altered gravity, as we have found for *NFL*, could be in agreement with this hypothesis. The use of a global transcriptomic study for this finding, as described in the paper, supports that this is a general mechanism, not restricted to specialized cells or tissues. Therefore, the observation of *NFL* expression in plant cells opens the possibility IFs play a role in signal transduction mechanisms of tropisms.

4.6 Conclusions

Our experiments on the International Space Station to study the interaction between phototropism and gravitropism revealed several novel and interesting genes that are involved in these tropism pathways. All five genes studied had some effects on blue/red light phototropism and gravitropism under some conditions. RT-PCR analysis of WT seedlings during blue-light-based phototropism added evidence that a gene (*NFL*) encoding an intermediate filament protein plays a role in the mechanisms of tropisms. These studies show the utility of spaceflight experiments in making discoveries in the fundamental biology of plants, which will be needed to fully optimize plant growth in bioregenerative life support systems in space missions. Future projects will examine the distribution of the NFL proteins in samples grown in space or in studies with microgravity analogues (such as clinostats; Kiss et al., 2019) in order to refine our understanding of intermediate filaments in gravity-dependent mechanisms in plants.

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Figure Captions

Fig. 1. Gravitropism experiments with the *Arabidopsis thaliana* WT, Columbia ecotype, and single gene *MTS*, *TMP*, *SGR*, *NGL*, or *ABC* knockout mutant seedlings that were (A, C) light-grown or (B, D) dark-grown prior the experiments. Graphs (A, B) show median shoot curvatures over time after 90° reorientation. Graphs (C, D) show median root curvatures over time after 90° reorientation. Mutant abbreviations indicate significant differences by pair-wise comparisons to WT, Wilcoxon rank sum tests. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, ° $P < 0.09$; each genotype $n = 61-77$.

Fig. 2. The unidirectional blue-light phototropism experiments with the WT (Columbia), and single gene *MTS*, *TMP*, *SGR*, *NGL*, or *ABC* knockout mutant seedlings that were (A, C) light-grown or (B, D) dark-grown prior the experiments. Graphs (A, B) show median shoot curvatures over time after experiment start. Graphs (C, D) show median root curvatures over time after experiment start. Mutant abbreviations indicate significant differences by pair-wise comparisons to WT, Wilcoxon rank sum tests. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, ° $P < 0.09$; each genotype $n = 61 - 75$.

Fig. 3. Images of light-grown seedlings used in the tropism time course studies. The response of the shoots of *nfl* mutant seedlings was reduced and delayed compared to WT seedlings at 48 h in both the gravitropism and blue-light phototropism time course studies. Arrows indicate the direction of gravity, and blue bulbs indicate the direction of the light source. Space between grid lines in the Petri dishes are 1.3 cm.

Fig. 4. The unidirectional red-light phototropism experiments with the WT (Columbia), and single gene *MTS*, *TMP*, *SGR*, *NGL*, or *ABC* knockout mutant seedlings that were (A, C) light-grown or (B, D) dark-grown prior the experiments. Graphs (A, B) show median & IQR shoot curvatures over time after experiment start. Graphs (C, D) show median & IQR root curvatures 24 h after experiment start. Mutant abbreviations indicate significant differences by pair-wise comparisons to WT, Wilcoxon rank sum tests. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, ° $P < 0.09$, each genotype $n = 60 - 68$.

Fig. 5. Changes in normalized RNA expression (mean \pm SE) of *MTS*, *TMP*, *SGR*, *NFL*, and *ABC* genes over the blue light treatment experiment in the WT *Arabidopsis thaliana* light-grown seedlings. $\Delta\Delta C_t$ were normalized with the *ACT8* gene. Different letters indicate significant difference, ANOVAI tests, $n = 3$.

TABLES

Table 1. Five genes of interest detected by differential expression in spaceflight studies. *Arabidopsis thaliana* knockout mutant gene functions are indicated. Differential gene expression analysis and adjusted p-value obtained from RNASeq analysis of spaceflight-flown samples from the space experiment (microgravity versus onboard 1g control). For details, see Vandenbrink et al. (2019).

Locus	Stock number	Abbreviation	Description	Log-2 Fold Change	Adjusted P-Value
AT1G22270	SALK_139799	<i>mts</i>	methyltransferase subunit	-22.62	4.76E-04
AT4G06534	SALK_068807	<i>tmp</i>	transmembrane protein	-22.09	7.79E-10
AT4G37650	SALK_002744	<i>sgr7</i>	transcription factor; affects shoot gravitropism	26.36	2.61E-05
AT5G25070	SALK_101138	<i>nfl</i>	neurofilament light protein	-23.51	6.27E-05
AT5G53650	SALK_049045	<i>abc</i>	ABC transporter A family protein	-7.33	2.00E-02

Table 2. Comparisons for shoot and root growth between knock-out mutants and wild-type *Arabidopsis thaliana* (Columbia) for light-grown and dark-grown seedlings in the ground-based **gravitropism experiments**.

Variable	Knock out mutant	Growth conditions	Results by Wilcoxon two-sample rank sum test, p-value						
			Time, h						
			0.5	2	4	8	24	48	72
Shoot growth	<i>mts</i>	Light	ns	ns	<i>mts</i> > WT*	ns	ns	ns	ns
		Dark	<i>mts</i> > WT***	ns	ns	ns	<i>mts</i> < WT*	<i>mts</i> < WT*	<i>mts</i> < WT*
	<i>tmp</i>	Light	ns	ns	ns	ns	ns	<i>tmp</i> > WT**	<i>tmp</i> > WT**
		Dark	ns	ns	ns	ns	ns	ns	ns
	<i>sgr</i>	Light	ns	<i>sgr</i> < WT*	ns	ns	ns	<i>sgr</i> > WT*	<i>sgr</i> > WT**
		Dark	<i>sgr</i> > WT***	ns	ns	ns	<i>sgr</i> < WT*	<i>sgr</i> < WT*	<i>sgr</i> < WT**
	<i>nfl</i>	Light	ns	ns	ns	<i>nfl</i> > WT***	<i>nfl</i> > WT*	<i>nfl</i> > WT*	ns
		Dark	<i>nfl</i> > WT***	<i>nfl</i> > WT**	ns	ns	<i>nfl</i> < WT***	<i>nfl</i> < WT**	<i>nfl</i> < WT***
	<i>abc</i>	Light	ns	ns	ns	ns	ns	<i>abc</i> > WT***	<i>abc</i> > WT***
		Dark	<i>abc</i> > WT***	<i>abc</i> > WT***	<i>abc</i> > WT*	ns	ns	ns	<i>abc</i> < WT*
Root growth	<i>mts</i>	Light	ns	<i>mts</i> < WT*	ns	ns	ns	ns	ns
		Dark	ns	ns	ns	ns	ns	ns	ns
	<i>tmp</i>	Light	ns	ns	ns	ns	ns	ns	ns
		Dark	ns	ns	ns	ns	ns	ns	<i>tmp</i> < WT ^o
	<i>sgr</i>	Light	ns	<i>sgr</i> < WT**	<i>sgr</i> < WT*	<i>sgr</i> < WT ^o	ns	ns	ns
		Dark	ns	ns	ns	ns	ns	ns	ns
	<i>nfl</i>	Light	ns	ns	ns	ns	ns	ns	ns
		Dark	ns	<i>nfl</i> < WT***	<i>nfl</i> < WT*	ns	<i>nfl</i> < WT*	ns	ns
	<i>abc</i>	Light	<i>abc</i> < WT*	<i>abc</i> < WT***	<i>abc</i> < WT***	<i>abc</i> < WT**	<i>abc</i> < WT*	ns	ns
		Dark	ns	ns	ns	ns	ns	ns	ns

Notes: ns – not significant, ^oP<0.06, * P<0.05, ** P <0.01, *** P<0.001, WT – wild type, *mts*, *sgr*, *nfl*, *abc* – single gene knockout mutants, *tmp* – multigene knockout mutant.

Table 3. Comparisons for shoot and root growth between knock-out mutants and wild-type *Arabidopsis thaliana* (Columbia) for light-grown and dark-grown seedling in the ground-based unidirectional **blue-light phototropism** experiments

			Results by Wilcoxon two-sample rank sum test, p-value							
Variable	Knockout mutant	Growth conditions	Time, h							
			0.5	1	2	4	8	24	36	48
Shoot growth	<i>mts</i>	Light	ns	ns	ns	ns	ns	ns	<i>mts</i> >WT***	<i>mts</i> >WT*
		Dark	ns	ns	ns	ns	ns	ns	ns	ns
	<i>tmp</i>	Light	<i>tmp</i> <WT**	<i>tmp</i> <WT**	<i>tmp</i> <WT**	<i>tmp</i> <WT***	<i>tmp</i> <WT ⁰	<i>tmp</i> >WT*	<i>tmp</i> >WT*	ns
		Dark	<i>tmp</i> <WT**	<i>tmp</i> <WT***	<i>tmp</i> <WT**	<i>tmp</i> <WT***	<i>tmp</i> <WT***	<i>tmp</i> <WT***	<i>tmp</i> <WT***	<i>tmp</i> <WT***
	<i>sgr</i>	Light	ns	ns	ns	<i>sgr</i> >WT ⁰	ns	<i>sgr</i> >WT***	<i>sgr</i> >WT***	<i>sgr</i> >WT***
		Dark	ns	<i>sgr</i> <WT*	<i>sgr</i> <WT***	ns	ns	ns	ns	ns
	<i>nfl</i>	Light	ns	ns	ns	ns	ns	<i>nfl</i> >WT***	<i>nfl</i> >WT**	<i>nfl</i> >WT***
		Dark	ns	ns	<i>nfl</i> <WT*	<i>nfl</i> <WT**	<i>nfl</i> <WT*	<i>nfl</i> <WT***	<i>nfl</i> <WT***	<i>nfl</i> <WT***
	<i>abc</i>	Light	ns	ns	<i>abc</i> <WT*	<i>abc</i> <WT*	ns	<i>abc</i> >WT***	<i>abc</i> >WT***	<i>abc</i> >WT***
		Dark	ns	ns	ns	<i>abc</i> <WT ⁰	ns	ns	<i>abc</i> <WT***	<i>abc</i> <WT*
Root growth	<i>mts</i>	Light	ns	ns	ns	<i>mts</i> >WT*	<i>mts</i> >WT*	<i>mts</i> >WT***	<i>mts</i> >WT***	<i>mts</i> >WT**
		Dark	ns	ns	ns	ns	ns	ns	ns	ns
	<i>tmp</i>	Light	<i>tmp</i> <WT*	ns	ns	ns	ns	ns	ns	ns
		Dark	ns	ns	<i>tmp</i> <WT**	<i>tmp</i> <WT*	<i>tmp</i> <WT***	<i>tmp</i> <WT***	<i>tmp</i> <WT***	<i>tmp</i> <WT***
	<i>sgr</i>	Light	<i>sgr</i> <WT*	ns	ns	ns	<i>sgr</i> >WT*	<i>sgr</i> >WT***	<i>sgr</i> >WT***	<i>sgr</i> >WT***
		Dark	ns	<i>sgr</i> >WT**	ns	<i>sgr</i> >WT*	<i>sgr</i> >WT*	<i>sgr</i> >WT*	ns	<i>sgr</i> >WT*
	<i>nfl</i>	Light	ns	ns	ns	ns	ns	<i>nfl</i> >WT*	<i>nfl</i> >WT**	<i>nfl</i> >WT***
		Dark	ns	<i>nfl</i> >WT*	ns	ns	ns	ns	ns	ns
	<i>abc</i>	Light	ns	ns	ns	<i>abc</i> >WT*	<i>abc</i> >WT**	<i>abc</i> >WT***	<i>abc</i> >WT***	<i>abc</i> >WT***
		Dark	ns	ns	ns	ns	ns	ns	ns	ns

Notes: ns – not significant, * P<0.05, ** P<0.01, *** P<0.001, WT – wild type, *mts*, *sgr*, *nfl*, *abc* – single gene knockout mutants. *tmp* – multigene knockout mutant.

Table 4. Comparisons for shoot and root growth between knock-out mutants and wild-type *Arabidopsis thaliana* (Columbia) for light-grown and dark-grown seedling in the ground-based unidirectional **red-light phototropism** experiments.

Knockout mutant	Growth conditions	Shoot growth 24 h, Wilcoxon two-sample rank sum test, P-value	Root growth 24 h, Wilcoxon two-sample rank sum test, P-value
<i>mts</i>	Light	ns	ns
	Dark	ns	<i>mts</i> > WT*
<i>tmp</i>	Light	ns	ns
	Dark	<i>tmp</i> < WT***	ns
<i>sgr</i>	Light	<i>sgr</i> < WT ^o	ns
	Dark	ns	<i>sgr</i> > WT**
<i>nfl</i>	Light	ns	<i>nfl</i> < WT*
	Dark	<i>nfl</i> < WT*	<i>nfl</i> > WT**
<i>abc</i>	Light	ns	ns
	Dark	<i>abc</i> < WT*	ns

Notes: ns – not significant, ^oP = 0.062, * P<0.05, ** P<0.01, *** P<0.001, WT – wild type, *mts*, *sgr*, *nfl*, *abc* – single gene knockout mutants. *tmp* – multigene knockout mutant.

Table 5. Effects of *MTS*, *TMP*, *SGR*, *NGL*, and *ABC* genes on *Arabidopsis thaliana* shoot and root curvature from light-grown (LG) and dark-grown (DG) seedlings at specific time points of the unidirectional blue light (BLUE), red (RED), and gravitropism (GRAV) experiments

Gene	Experiment	Shoot curvature		Root curvature	
		LG	DG	LG	DG
<i>MTS</i>	BLUE	Attenuate 1-48 h	Stimulate 4-8 h	Attenuate 4-48 h	-
	RED	-	-	Attenuate 24 h	-
	GRAV	Stimulate 48-72 h	-	Stimulate 0.5-8 h	Stimulate 0.5, 4-8h
<i>TMP</i>	BLUE	Attenuate 1-8 h	Stimulate 0.5 – 48 h	Attenuate 8 h	-
	RED	Attenuate 24 h	-	-	-
	GRAV	-	-	Attenuate 72 h	Stimulate 4-8, 72 h
<i>SGR</i>	BLUE	Attenuate 2-24 h	Stimulate 4-48 h	Attenuate 4-24 h	-
	RED	-	-	Attenuate 24 h <i>P</i> =0.053	-

	GRAV	Stimulate 0.5-48 h	Stimulate 24-72 h	Stimulate 2 h Attenuate 72 h	-
<i>NFL</i>	BLUE	Attenuate 1-8 h	Stimulate 4-48 h	Attenuate 8-24 h	-
	RED	Attenuate 24h	-	Attenuate 24h	-
	GRAV	Stimulate 24-72 h	Stimulate 24 h	Stimulate 2 h	-
<i>ABC</i>	BLUE	Attenuate 1-36 h	Stimulate 4-48 h	Attenuate 8-48 h	Stimulate 0.5 h
	RED	-	-	Attenuate 24h	-
	GRAV	Attenuate 8 h Stimulate 48-72 h	Stimulate 24-72 h	Attenuate 48-72 h	-

Supplementary Table S1. Primers used for qPCR SYBR Green assays and PCR product melting temperatures.

Gene	Primer name	Sequence 5' – 3'	Observed <i>A.thaliana</i> T _m , °C	Design
<i>MTS</i>	mts-1F	CGCGGTGCTGAAATCAGATG	83.62	Primer Blast
	mts-1R	GTCCAGTCTCAGGGCAAACA		Primer Blast
<i>TMP</i>	tmp-10F	GGGTGGACAAGGTTTCGGATT	85.7	Primer Blast
	tmp-10R	ACACCAGGATCTTCGCTTCC		Primer Blast
<i>SGR</i>	sgr-6F	GGCAAACGGAGCAATCTTGG	82.13	Primer Blast
	sgr-6R	GAGGCGTGTCGTCTGATCTT		Primer Blast
<i>NFL</i>	nfl-7F	AAGTGGCTGCATCAACGAGA	77.25	Primer Blast
	nfl-7R	GCTTTTTTCGAGCTCAGCGTT		Primer Blast
<i>ABC</i>	abc-9F	GTTTTGATTCGCGGTTGGTCT	78.99	Primer Blast
	abc-9R	CAGCTCCGGCGAAGAAAGAA		Primer Blast
<i>ACT8</i>	ACT8f	ATGAAGATTAAGGTCGTGGCA	81.78	(Morita et al. 2006)
	ACT8r	TCCGAGTTTGAAGAGGCTAC		(Morita et al. 2006)

Supplementary Table S2. Shoot and root growth in single gene knock-out mutants and wild-type *Arabidopsis thaliana* (Columbia) for light-grown and dark-grown seedlings in the gravitropism experiments.

Genotype	Time,h	Light- grown				Dark- grown			
		Shoot growth, mm		Root growth, mm		Shoot growth, mm		Root growth, mm	
		Median	IQR	Median	IQR	Median	IQR	Median	IQR
WT	0.5	0.041	0.11275	0.134	0.312	0.0285	0.1815	0.0395	0.15575
<i>mts</i>	0.5	0.03	0.0985	0.135	0.28025	0.142	0.22075	0.04	0.2355
<i>tmp</i>	0.5	0.0215	0.1025	0.11	0.132	0.035	0.1865	0.025	0.202
<i>sgr</i>	0.5	0.029	0.112	0.09	0.171	0.1555	0.22775	0.045	0.149
<i>nfl</i>	0.5	0.0665	0.12125	0.01175	0.28825	0.003	0.215	0.0075	0.184
<i>abc</i>	0.5	0.0445	0.103	0.0745	0.13175	0.177	0.2895	0.055	0.15575
WT	2	0.086	0.225	0.4035	0.417	0.457	0.28275	0.178	0.28575
<i>mts</i>	2	0.089	0.177	0.291	0.259	0.4615	0.52025	0.176	0.273
<i>tmp</i>	2	0.0525	0.1705	0.277	0.324	0.449	0.3565	0.122	.3015
<i>sgr</i>	2	0.038	0.184	0.256	0.249	0.539	0.4925	0.188	0.285
<i>nfl</i>	2	0.1225	0.23625	0.4185	0.393	0.581	0.317	0.0575	0.2965
<i>abc</i>	2	0.0835	0.145	0.1775	0.23225	0.631	0.3265	0.154	0.241
WT	4	0.09	0.185	0.4625	0.792	0.624	0.287	0.2575	0.028425
<i>mts</i>	4	0.122	0.148	0.485	0.534	0.6085	0.30525	0.271	0.2785
<i>tmp</i>	4	0.0575	0.143	0.495	0.577	0.55	0.43475	0.209	0.308
<i>sgr</i>	4	0.083	0.153	0.333	0.315	0.7305	0.41075	0.236	0.245
<i>nfl</i>	4	0.142	0.2525	0.516	0.43975	0.612	0.301	0.191	0.1995
<i>abc</i>	4	0.1035	0.17325	0.255	0.3705	0.768	0.3665	0.2395	0.256
WT	8	0.159	0.207	0.81	1.0835	0.97	0.459	0.3985	0.25625
<i>mts</i>	8	0.1715	0.203	0.942	1.054	0.9015	0.43175	0.38	0.2355
<i>tmp</i>	8	0.1505	0.22725	0.894	1.221	0.9265	0.577325	0.396	0.3375
<i>sgr</i>	8	0.163	0.155	0.588	0.576	1.032	0.5085	0.401	0.231
<i>nfl</i>	8	0.238	0.22375	0.745	0.538	0.903	0.438	0.336	0.29375
<i>abc</i>	8	0.176	0.14675	0.575	0.56	1.006	0.4625	0.357	0.212
WT	24	0.5615	0.436	2.604	2.2895	2.65	1.127	1.069	0.43475
<i>mts</i>	24	0.4895	0.4525	2.9095	2.61925	2.302	1.0075	1.067	0.4415
<i>tmp</i>	24	0.6235	0.51775	2.435	2.62	2.4495	1.56275	0.926	0.5495
<i>sgr</i>	24	0.483	0.464	2.259	1.327	2.334	0.96175	1.063	0.465
<i>nfl</i>	24	0.692	0.51275	2.2545	1.07875	2.179	0.679	0.864	0.507
<i>abc</i>	24	0.6	0.39825	2.0075	1.4735	2.442	0.8065	0.9685	0.568
WT	48	1.312	0.77225	4.721	3.55	4.3095	1.76275	1.724	0.906
<i>mts</i>	48	1.281	0.6365	5.2545	3.768	3.888	1.521	1.748	0.814
<i>tmp</i>	48	1.563	1.16	4.3405	4.10875	4.1315	2.6325	1.605	0.751
<i>sgr</i>	48	1.589	0.748	4.472	1.863	3.868	1.64025	1.876	0.921
<i>nfl</i>	48	1.524	0.7895	3.95	1.71725	3.7675	1.16875	1.68	0.778
<i>abc</i>	48	1.7355	0.65625	4.28	2.20475	4.151	1.1195	1.615	0.67075
WT	72	2.165	1.138	5.994	4.5945	5.875	1.9975	2.458	1.11
<i>mts</i>	72	1.98	0.918	6.59	4.563	5.1335	1.93175	2.378	1.161
<i>tmp</i>	72	2.558	1.635	5.6515	4.55325	5.449	3.504	2.092	1.001
<i>sgr</i>	72	2.509	0.969	5.897	2.346	5.194	2.10975	2.521	1.457
<i>nfl</i>	72	2.3235	1.276	5.776	1.893	4.916	1.835	2.317	1.0085
<i>abc</i>	72	2.977	0.92025	5.69755	2.42575	5.283	1.4755	2.328	1.16875

Supplementary Table S3. Shoot and root growth in knock-out mutants and wild-type *Arabidopsis thaliana* (Columbia) for light-grown and dark-grown seedlings in unidirectional blue-light phototropism experiments.

Genotype	Time,h	Light- grown				Dark- grown			
		Shoot growth, mm		Root growth, mm		Shoot growth, mm		Root growth, mm	
		Median	IQR	Median	IQR	Median	IQR	Median	IQR
WT	0.5	0.0085	0.11275	0.2745	0.4955	0.086	0.221	0.087	0.304
<i>mts</i>	0.5	0.046	0.146	0.188	0.3435	0.13	0.342	0.0565	0.2975
<i>tmp</i>	0.5	-0.041	0.2145	0.0985	0.28875	0.043	0.2715	0.048	0.24975
<i>sgr</i>	0.5	0.044	0.169	0.132	0.227	0.0535	0.2995	0.07	0.271
<i>nfl</i>	0.5	0.028	0.217	0.245	0.274	0.086	0.31	0.0795	0.2555
<i>abc</i>	0.5	-0.014	0.1655	0.139	0.28675	0.052	0.2455	0.067	0.2445
WT	1	0.0625	0.25425	0.1865	0.42425	0.108	0.2125	0.081	0.248
<i>mts</i>	1	0.099	0.198	0.227	0.4	0.17	0.296	0.1465	0.294
<i>tmp</i>	1	-0.01	0.177	0.095	0.388	0.029	0.2185	0.061	0.294
<i>sgr</i>	1	0.0725	0.19925	0.261	0.3585	0.21	0.327	0.1615	0.30125
<i>nfl</i>	1	0.1105	0.20925	0.273	0.328	0.159	0.3055	0.1655	0.3235
<i>abc</i>	1	0.042	0.162	0.277	0.374	0.085	0.294	0.129	0.2865
WT	2	0.124	0.21175	0.3655	0.475	0.208	0.283	0.174	0.298
<i>mts</i>	2	0.128	0.1745	0.387	0.4905	0.2225	0.29725	0.158	0.26
<i>tmp</i>	2	0.0505	0.18975	0.303	0.37175	0.137	0.2765	0.16	0.294
<i>sgr</i>	2	0.0925	0.2395	0.43	0.372	0.348	0.283	0.2415	0.35475
<i>nfl</i>	2	0.183	0.264	0.376	0.417	0.099	0.317	0.1735	0.2605
<i>abc</i>	2	0.057	0.273	0.421	0.419	0.191	0.291	0.149	0.3
WT	4	0.02025	0.26025	0.5165	0.6035	0.392	0.354	0.25	0.324
<i>mts</i>	4	0.191	0.287	0.832	0.7125	0.305	0.292	0.2735	0.34775
<i>tmp</i>	4	0.0285	0.259	0.474	0.5185	0.169	0.2755	0.159	0.322
<i>sgr</i>	4	0.127	0.193	0.796	0.7575	0.382	0.402	0.374	0.413
<i>nfl</i>	4	0.165	0.23225	0.623	0.873	0.2885	0.348	0.243	0.34925
<i>abc</i>	4	0.107	0.227	0.748	0.69	0.287	0.379	0.244	0.302
WT	8	0.285	0.314	0.927	1.017	0.582	0.3875	0.476	0.296
<i>mts</i>	8	0.351	0.47	1.522	1.3165	0.524	0.388	0.493	0.4515
<i>tmp</i>	8	0.17	0.31275	0.907	0.9815	0.412	0.3335	0.3015	0.275
<i>sgr</i>	8	0.269	0.298	1.375	1.62375	0.668	0.408	0.569	0.334
<i>nfl</i>	8	0.32	0.364	1.152	1.415	0.4425	0.346	0.421	0.32975
<i>abc</i>	8	0.3345	0.28275	1.431	1.36	0.523	0.3275	0.451	0.3285
WT	24	0.632	0.5865	2.295	2.767	0.935	0.5765	1.202	0.656
<i>mts</i>	24	0.351	0.6455	4.758	3.287	0.735	0.54925	1.204	0.89625
<i>tmp</i>	24	0.8795	0.696	2.889	2.527	0.55	0.474	0.7475	0.69875
<i>sgr</i>	24	1.066	0.702	4.055	4.119	0.892	0.5125	1.3575	0.72175
<i>nfl</i>	24	1.065	0.777	3.904	4.0725	0.6565	0.451	1.0395	0.6435
<i>abc</i>	24	1.1065	0.552	4.541	2.6555	0.858	0.453	1.123	0.5545
WT	36	0.963	0.62	4.1175	3.59425	1.119	0.771675	1.6205	0.89675
<i>mts</i>	36	1.143	0.81675	6.697	4.352	1.012	0.632	1.5605	1.2215
<i>tmp</i>	36	1.135	0.554	4.257	3.70575	0.7535	0.44325	0.9535	0.83675
<i>sgr</i>	36	1.3025	0.8905	6.403	5.39025	1.08	0.541	1.798	1.00125
<i>nfl</i>	36	1.3285	1.123	6.241	4.8715	0.772	0.52625	1.501	0.87425
<i>abc</i>	36	1.4575	0.771	6.915	3.841	0.841	0.4515	1.361	0.837

WT	48	1.225	0.7695	6.337	3.947	1.18	0.71	1.884	1.0675
<i>mts</i>	48	1.484	0.9435	8.231	5.7185	1.059	0.751	2.242	1.804
<i>tmp</i>	48	1.5025	0.884	5.1506	4.6095	0.9185	0.546	1.376	1.09
<i>sgr</i>	48	1.712	1.3315	9.304	5.8195	1.119	0.5415	2.3295	1.1895
<i>nfl</i>	48	1.86	1.73	8.457	5.86475	0.8795	0.46575	2.137	1.246
<i>abc</i>	48	1.99	1.142	9.331	4.091	0.945	0.5875	1.933	1.3005

Supplementary table S4. Shoot and root growth in knock-out mutants and wild-type *Arabidopsis thaliana* (Columbia) for light-grown and dark-grown seedlings in unidirectional red-light phototropism experiments.

Genotype	Time, h	Light- grown				Dark- grown			
		Shoot growth, mm		Root growth, mm		Shoot growth, mm		Root growth, mm	
		Median	IQR	Median	IQR	Median	IQR	Median	IQR
WT	24	0.321	0.329	5.089	5.16025	1.797	1.014	0.6185	0.9325
<i>mts</i>	24	0.227	0.253	4.917	4.5335	1.595	1.5775	1.004	1.0985
<i>tmp</i>	24	0.284	0.3295	5.435	4.2915	1.101	1.013	0.4965	0.66075
<i>sgr</i>	24	0.265	0.243	5.002	4.34125	1.582	0.993	1.148	1.022
<i>nfl</i>	24	0.278	0.209	3.583	4.2075	1.47	1.259	0.9555	0.64475
<i>abc</i>	24	0.262	0.31	5.874	5.233	1.406	1.37275	0.759	1.09425

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