Postdoc Fellowships for non-EU researchers

Final Report

Name	Andrés Ritter
Selection	2011
Host institution	Gent University
Supervisor	Professor Dirk Inzé and Professor Alain Goossens
Period covered by this report	from 15/02/2012 to 15/09/2013
Title	Characterization of the molecular mechanisms of plant growth regulation by jasmonate oxylipins in Arabidopsis thaliana

1. Objectives of the Fellowship (1/2 page)

Light is probably the most important environmental factor shaping almost every aspect of plant development including germination, seedling (de)etiolation, flowering time, circadian rhythm and avoidance responses to shade or excessive light. Light signals are tightly interconnected with phytohormonal pathways that play a key role in the translation of environmental light cues into the correct developmental program to ensure plant growth and survival. For example, shade growing plants will balance resources toward fast growth and limit the investment in chemical defence responses. At the molecular level, this process results in the repression of the jasmonate (JA)-dependent signalling pathway, since JA induces defence and slows growth, which is in direct opposition to shade-induced responses. JA-responsive gene expression is tightly controlled by a set of transcription factors which are inhibited in a JA unelicited state by a core transcriptional repressor complex composed of the JAsmonate Zim domain proteins (JAZ), the Novel Interactor of JAZ proteins (NINJA) and the co-repressor TOPLESS (TPL) (Pauwels and Goossens, 2011). In this context the scientific objectives of this fellowship were to characterize the *Arabidopsis thaliana* transcription factors FAR1 RELATED SEQUENCE 7 (FRS7) and 12 (FRS12) that we identified as physically interacting with the JA-related NINJA-TPL co-repressor complex. Arabidopsis lines presenting altered expression of *FRS7* and *FRS12* show different red light hypocotyl elongation phenotypes, suggesting the implication of FRS7/12 in red light signalling. RNA-Seq analysis revealed that *FRS12* overexpression leads to repression of genes involved in red light signalling and circadian rhythm, in particular members of the *Phytochrome Interactor Factor (PIF)* transcription factor family that regulate plant development in response to light and photoperiodism. Expression of the FRS7/12 complex is diurnally regulated and peaks at night, which is negatively correlated with the expression of a large portion of its target genes, including *PIF4*. Therefore, we postulate that the FRS7/12 protein complex constitutes a novel light regulated machinery that controls several aspects of circadian regulation of plant growth.

2. Methodology in a nutshell (1/2/ page)

This project developed a reverse genetic approach in *Arabidopsis thaliana* by integrating transcriptome and protein interactome techniques.

All Arabidopsis plants used in this study were in the Columbia ecotype background and all plant transformations were carried out by floral dip using *Agrobacterium tumefaciens* strain C58C1.

Tandem affinity purification (TAP) experiment was carried-out in *Arabidopsis thaliana* PSB-D cells to identify new protein interactors of NINJA. TAP consists of the co-isolation of a specific tagged protein (bait) and its interactive partners (preys) under native conditions through two consecutive affinity-purification steps. Combined with mass spectrometry, the TAP strategy allows the identification of proteins interacting with the given target protein. We used previously established TAP methods that had already allowed identifying new components of the JA signalling machinery such as NINJA itself (Pauwels et al., 2010; Van Leene et al., 2011). Identified protein interactors were further confirmed using the yeast two hybrid (Y2H) assay.

Transient Expression Assays in Tobacco Protoplasts (TEA) were performed as previously described (Vanden Bossche et al., 2013). Accordingly, FRS7 and FRS12 were fused N-terminally to the GAL4DBD and co-transfected with a reported plasmid that drives the expression of the firefly luciferase under regulation of the prormoter *UAS* (*prUAS*).

For RNA-Seq analysis, samples from Arabidopsis seedlings expressing *Pro35S:FRS12-GR* and *Pro35S:GFP-GR* RNA were processed by first preparing a Trueseq RNA-Seq library (Illumina) and then sequenced at 30 million reads depth at 50bp single read using Illumina HiSeq 2000 technology at GATC Biotech Ltd - Germany. Reads were subsequently mapped to the TAIR10 version of the *Arabidopsis* genome using GSNAPv2 (Wu and Nacu, 2010) allowing a maximum of 5 mismatches. Statistical analyses were carried on a pipeline implemented in edgeR (Robinson et al., 2010).

3. Results (6-8 pages)

FRS7 and FRS12 Are Novel Interactors of NINJA

Recently, NINJA was shown to be indispensable for repressing JA signaling in the root, allowing normal root cell elongation by a mechanism in which MYC2 either has no role or is not the only TF repressed by interaction with NINJA. Therefore, NINJA was fused carboxy-terminally to a TAP-tag and expressed under the *CaMV35S* promoter (*Pro35S*) in *Arabidopsis* cell cultures. This TAP method expanded the protein identifications from a previous analysis with additional known components of the core JA signaling module (Fig. 1 A). These included the JAZ proteins JAZ2 and JAZ12, the co-repressor TPL, which are all direct interactors of NINJA, as well as transcriptional activators of JA-signaling, the bHLH factors MYC2, MYC3 and MYC4, which interact directly with the JAZ proteins. In addition, this TAP experiment identified new potential interactors of NINJA, such as the hitherto uncharacterized protein FRS12, encoded by the locus AT5G18960. FRS12 belongs to a family of light signaling related transposase-derived TFs that encompasses 14 members in *Arabidopsis* (Lin and Wang, 2004) (Fig. 1B). FAR-RED ELONGATED HYPOCOTHYL 3 (FHY3) and its paralog FAR-RED IMPAIRED RESPONSE 1 (FAR1) are the two best characterized members of the FRS family, being essential transcriptional activators acting downstream of phyA to regulate photomorphogenic development (Lin et al., 2007). A systematic yeast two-hybrid (Y2H) analysis between with FRS12 against all proteins identified in the NINJA-TAP revealed that FRS12 binds directly to NINJA (Fig. 1B). To evaluate whether additional members of the FRS family could also directly interact with NINJA, we carried out a Y2H experiment with 7 other members of the FRS family, including FHY3 and FAR1. This analysis revealed that NINJA interacts specifically with FRS12 and its close homolog FRS7 (Fig. 1 D). These results establish FRS7 and FRS12 as new interactor proteins of NINJA.

FRS7 and FRS12 Act as Nuclear Localized Transcriptional Repressors

To determine the subcellular localization of the FRS7 and FRS12 proteins, carboxy-terminal GFP fusions of these proteins were expressed in *Arabidopsis* plants. Laser scanning confocal images of primary root tip cells showed distinctive nuclear signals for both FRS7-GFP and FRS12-GFP constructs (Fig. 2A), defining FRS7 and FRS12 as nuclear localized proteins. NINJA has also been localized in the nucleus, where it recruits TPL to JAZ proteins in order to form a repressor complex (Pauwels et al., 2010). To further assess this link, we used the yeast three-hybrid (Y3H) system to demonstrate that NINJA was capable of bridging FRS12 to TPL (Fig. 2B). Subsequently, we determined the potential transcriptional regulatory activities of FRS7 and FRS12 in a transient expression assay in tobacco protoplasts, showing that both could repress firefly luciferase (*fLUC*) reporter gene expression (Fig. 2C-D). Taken together, these results suggest that FRS12 and FRS7 can recruit the NINJA-TPL co-repressor complex in the nucleus to repress gene transcription.

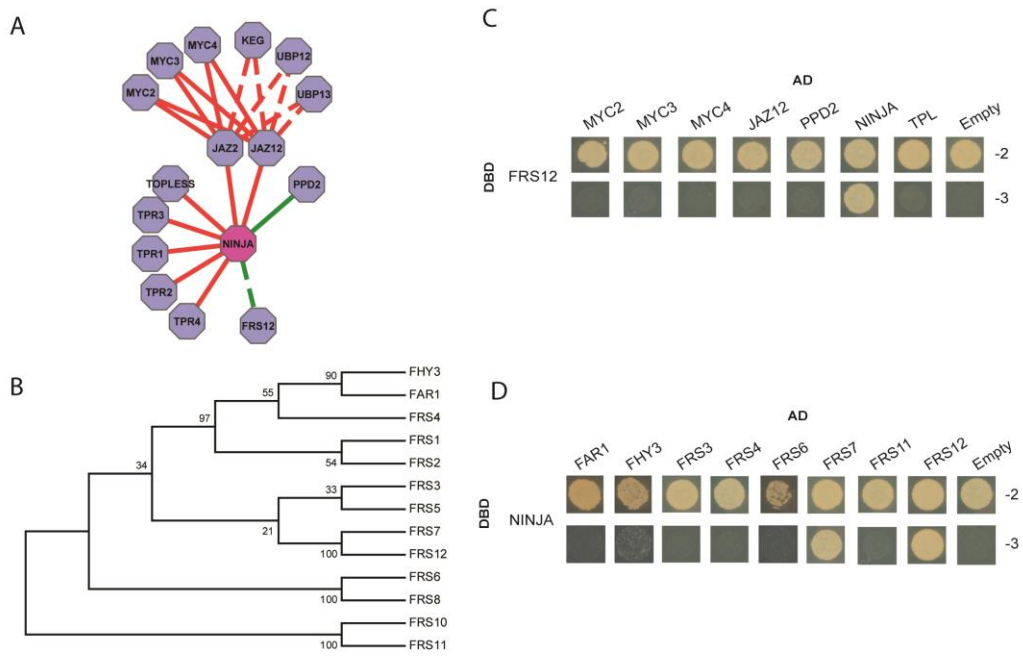

Figure 1. NINJA Interacts with the FRS12 Protein.
(A) Cytoscape protein interaction network build on NINJA-TAP results. Pink node indicate the protein bait (NINJA) and purple nodes the co-purified proteins. Red edges indicate JA signalling related proteins and green edges other processes. Solid edges indicate previously confirmed interactions whereas dashed lines show previously unkown interactions.
(B) Maximum-likelihood phylogenetic tree of the FAR1 RELATED SEQUENCE (FRS) family. Numbers above branches represent bootstrap values.
(C) Y2H experiment testing FRS12 fused to the GAL4BD as bait against identified NINJA-TAP interactors as preys fused to GAL4AD.
(D) Y2H screen testing the interaction of NINJA fused to GAL4BD with FRS family proteins fused to GAL4AD.

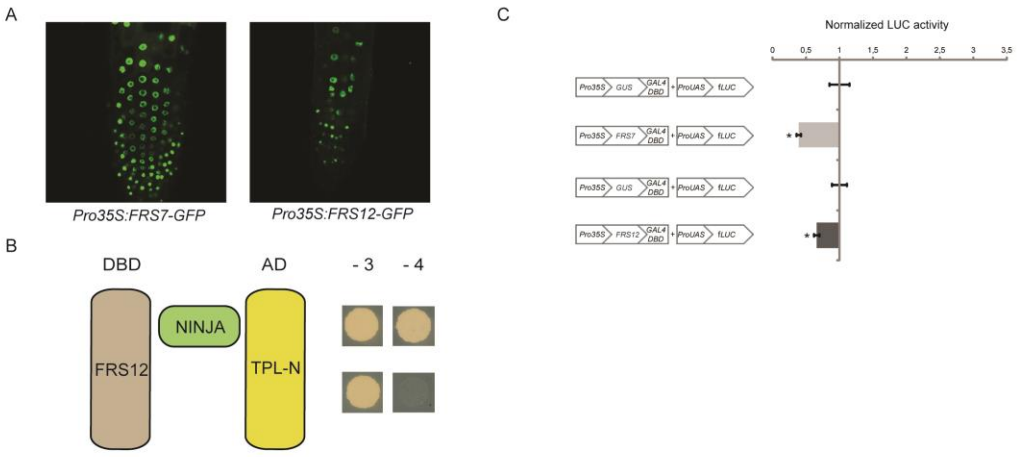

Figure 2. FRS7 and FRS12 Are Nuclearly Localized Transcriptional Repressors.
(A) Laser confocal microscope images of Arabidopsis primary root cells expressing *FRS7-GFP* (left) and *FRS12-GFP* (right).
(B) Y3H of FRS12 (fused to GAL4DB), NINJA and the N-terminal region of TOPLESS (fused to GAL4AD). Transformed yeasts were spotted in 10-fold dilutions on control medium (-3) or selective medium (-4). Upper and lower spots correspond to yeasts transformed with NINJA or the corresponding empty vector control, respectively.
(C) Transactivation assay in tobacco protoplasts co-transfected with a *ProUAS-fLUC* reporter construct, effector constructs (FRS12 and FRS7, fused to GAL4DBD) and a *Pro35S:rLUC* construct to normalize expression. Values for C and D represent the mean of 8 replicates relative to the mean expression of a *Pro35S:GUS-GAL4DBD* control that was adjusted to 1. Error bars represent the SE. Asterisks indicate statistically significant differences with respect to the *Pro35S:GUS-GAL4DBD* control (*p<0.05; **p<0.01, T-test).

FRS12 Represses Red Light and Photoperiodic Related Mechanisms

To examine the mechanism in which FRS12 is implicated, we generated transgenic *Arabidopsis* lines constitutively overexpressing *FRS12* fused carboxy-terminally to the hormone binding domain of the rat glucocorticoid receptor (GR) that translocates to the nucleus in the presence of dexamethasone (DEX). To identify the processes affected by *FRS12* overexpression in *Arabidopsis*, we carried out a RNA-Seq analysis. Long-day grown seedlings expressing *Pro35S:FRS12-GR1* and *Pro35S:GFP-GR* (control) were treated with 5 µM DEX and harvested at night time (ZT 23). Transcriptome comparison between the *Pro35S:FRS12-GR1* and Pro35S*:GFP-GR* lines revealed 351 differentially expressed genes (FDR<0.05 with a fold change > 2). Among these genes, 216 showed up-regulation whereas 135 were down-regulated.

GO enrichment analysis showed that *FRS12* overexpression resulted in the up-regulation of a number of stress related genes. These genes also grouped under phytohormone responsive terms related to stress responses such as JA, ethylene, or salicylic acid. A set of specialized (secondary) metabolism genes such as those from the camalexin biosynthesis were also enriched in the *Pro35S:FRS12-GR1* line. However, considering the repressor activity of the FRS12 protein, these genes were not further investigated in this study. Instead, we focused on the down-regulated genes, for which GO analysis highlighted red light signalling and photoperiodic processes among the most significantly enriched. Among the genes involved in red light signalling processes, we identified central regulators of photo- and skotomorphogenic development. The gene coding for *PIF4* displayed a more than 10-fold down-regulation and constituted the fifth most highly repressed gene of this study (Fig. 3A). This transcription factor has a key role in the promotion of etiolated plant growth, and this activity is reversed upon interaction with phytochrome in response to light (Leivar and Monte, 2014). Furthermore, *FRS12* overexpression also repressed the expression of *PRE1*, encoding a HLH transcriptional regulator that heterodimerizes with PIF4 to induce cell elongation and plant development in response to light (Hao et al., 2012). The activity of PIF4 is regulated at the transcriptional level by PIL1 and HFR1, that act in a co-ordinate matter to repress PIFs' activity and therefore induce photomorphogenesis (Luo et al., 2014). Both *PIL1* and *HFR1* were also repressed in the *Pro35S:FRS12-GR1* line. These results suggest the function of FRS12 as a light signalling regulator in Arabidopsis.

The second set of highly enriched GO terms of the down-regulated genes was related to circadian rhythm processes. This group also includes *PIF4* that promotes phytochrome and clock-regulated growth under photoperiodic conditions (Nusinow et al., 2011; Soy et al., 2014). Furthermore, also *GIGANTEA* (*GI*) was one of the most down-regulated genes in the *Pro35S:FRS12-GR1* line. This protein plays key functions as a circadian clock-controlled regulator of flowering time in Arabidopsis (Sawa and Kay, 2011). Other genes encoding for components of the circadian clock, such as the *PSEUDO RESPONSE REGULATOR7* (*APRR7)*, A*PRR3* and the single MYB transcription factor *REVEILLE7 (RVE7)*, were also repressed by *FRS12* overexpression (Harmer, 2009). These results suggest that FRS12 could act as a light signalling input into the circadian clock.

The expression patterns observed in the RNA-Seq analysis were evaluated by independent gene expression measurements through qPCR analysis in two independent *Pro35S:FRS12-GR* lines sampled at NT. The analysed genes included *PIF4*, *PIL1*, *APRR7*, *GI*, and *FLOWERING PROMOTING FACTOR 1-LIKE PROTEIN 1* (*FLP1)*. This analysis confirmed the accuracy of the RNA-Seq analysis and demonstrated the repressing effect of ectopic *FRS12* expression (Fig. 3B).

These results establish the role of FRS12 as a direct regulator of red light signalling, flowering time and photoperiodic processes.

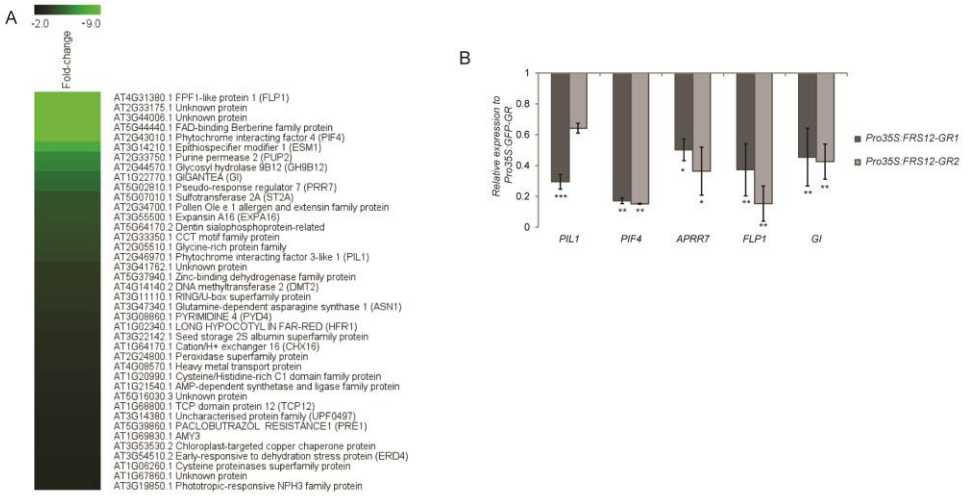

Figure 3. FRS12 Represses Red Light Signaling and Photoperiodic Related Processes.
(A) Heat map representing the top 40 down-regulated genes in the RNA-Seq analysis of the *Pro35S:FRS12-GR1* line.
(B) Gene expression determined by qPCR analysis in response to 4-h treatment of DEX at night at 21 hours zeitgeber time (ZT21). Two independent *Pro35S:FRS12GR* lines and a *Pro35S:GFP-GR* control line were grown for one week under long days, then treated with 5 μM of DEX and harvested at four hours after treatment (ZT21) for qPCR. Expression values of *PIL1*, *PIF4*, *APRR7*, *FLP1* and *GI* were evaluated in the *Pro35S:FRS12-GR1*, *Pro35S:FRS12-GR2* and *Pro35S:GFP-GR* lines and normalized to the expression of *UBC* (AT5G25760) and *PP2A* (At1g13320) as internal expression controls. Gene expression values of the *Pro35S:FRS12GR-1*, *Pro35S:FRS12GR-2* lines are represented relative to those of the *Pro35S:GFP-GR* control line that was adjusted to 1. Error bars represent the SE of three biological replicates. Asterisks indicate statistically significant differences with respect to the *Pro35S:GFP-GR* line (*p<0.05; **p<0.01; ***p<0.001, T-test).

The Expression of the FRS7/FRS12 Complex Is Regulated by the Circadian Clock

As shown above, our RNA-Seq results suggest that FRS7/FRS12 are involved in the circadian clock-controlled regulation of photoperiodic flowering in plants. An illustrative, independent link between FRS12 and its target genes was encountered when we consulted the DIURNAL and PHASER tools (http://diurnal.mocklerlab.org/) to monitor the circadian gene expression of the members of the FRS7/FRS12 complex in short-day grown Col-0 wild-type plants. *In silico* gene expression analysis using the PHASER tool showed that the majority (*i.e.* 102 genes) of the 135 genes repressed by *FRS12* overexpression display a circadian regulation (Fig. 4A). Furthermore the circadian regulation of most of these genes phased at day time or at the end of the night period. This trend was even more pronounced for those genes showing high repression (consisting in a log fold change higher than 2) in the *Pro35S:FRS12-GR1* line, such as the FRS12 direct gene targets *GI* or *PIF4* (Fig. 4B). Conversely, diurnal gene expression of *FRS7*/*FRS12* and their

interacting partner proteins (Fig. 2B) peaked at night time, suggesting their peak of activity occurs at this period of the day. Thus these results point to a negative correlation between the diurnal expression of the FRS7/FRS12 repressor complex and the expression of the FRS12-repressed genes.

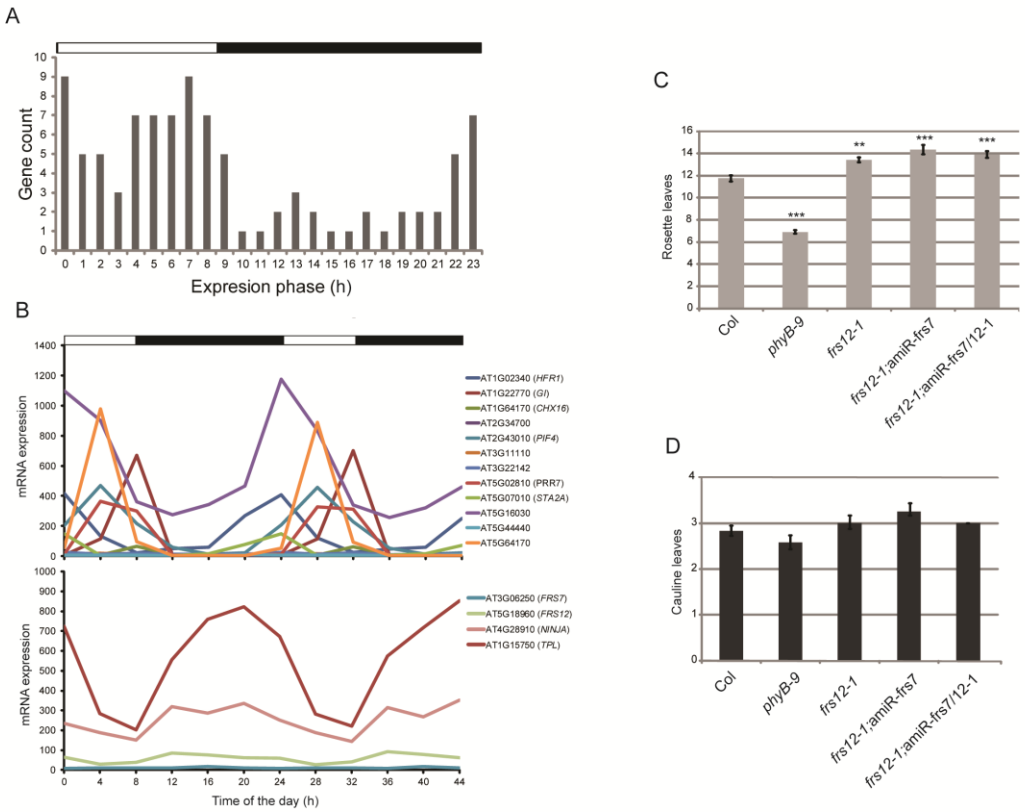

Figure 4. Expression of the FRS7/FRS12 Complex Is Regulated in a Circadian Manner.
(A) *In silico* analysis showing the counts of diurnal peaks of expression of the *Pro35S:FRS12-GR1* down-regulated genes in Col-0 wild-type *Arabidopsis* grown under short days.
(B) *In silico* analysis showing the circadian expression of the *Pro35S:FRS12-GR1* repressed genes presenting a log fold-change > 2 (upper panel) compared to the diurnal expression of the genes encoding the FRS7/FRS12 complex (lower panel).
(C) Long-day flowering time assay of *frs12-1*, *frs12-1*;amiR-frs7 and *frs12-1*;amiR-frs7/12-1 lines. The number of visible rosette leaves was recorded when the first flower bud opened. Error bars represent the SE of 15 biological replicates. Asterisks indicate statistically significant differences between Col-0 wild-type *Arabidopsis* and the indicated genotypes (*p<0.05; **p<0.01; ***p<0.001, T-test).
(D) Long-day flowering time assay of *frs12-1*, frs12-1;amiR-frs7 and *frs12-1*;amiR-frs7/12-1 lines. The number of visible cauline leaves was recorded when the first flower bud opened. Error bars represent the SE of 15 biological replicates. No statistically significant differences were observed between Col-0 wild-type and the tested genotypes (T-test).

FRS7 and FRS12 Mediate Light Regulation of Flowering Time and Hypocotyl Growth

Because of the above, we evaluated if *FRS7* and *FRS12* loss-of-function Arabidopsis lines were affected in their flowering time behavior, a process that is directly regulated by photoperiodism. Therefore, a *FRS12* null mutant, hereafter referred to as *frs12-1*, was independently transformed with three available artificial micro-RNA (amiR) constructs targeting the expression of *FRS7* or both *FRS7* and *FRS12*. These lines will be hereafter referred to as *frs12-1*;amiR-frs7, *frs12-1*;amiR-frs7/12-1, and *frs12-1*;amiR-frs7/12-2. Both *frs12-1* and *frs12-1;*amiR lines flowered significantly later at a stage of ~14 rosette leaves compared to wild-type plants that flowered at ~12 leaves (Fig. 4C). Furthermore, delayed flowering time was more accentuated in the *frs12-1;*amiR lines, suggesting the coordinated functions FRS7 and FRS12 in the

regulation of photoperiodic processes comprising flowering time. No significant differences were observed among the tested genotypes at the number cauline leaves (Fig. 4D).

In addition to circadian regulation, our results point to a possible involvement of FRS7/FRS12 in red light signaling pathways. Hence, we evaluated if *FRS7* and *FRS12* loss-of-function lines presented compromised sensitivity to red light. Therefore, the inhibition of hypocotyl elongation was measured in *frs12-1* and *frs12-1*;amiR lines grown in continuous Dark (cD), Red (cR) or Far Red light (cFR) sources. Seedlings of the *frs12-1* and *frs12-1*;amiR lines grew significantly shorter hypocotyls under cR compared to wild-type plants (Fig. 5A), suggesting that the loss-of-function of *FRS7* and *FRS12* results in partial red light hypersensitivity in *Arabidopsis*. No striking elongation differences were observed among lines when grown in cFR (Fig. 5B). In contrast, cD grown *frs12-1*, and two of the *frs12-1*;amiR lines showed a significantly increased hypocotyl elongation compared to wild type, attesting for partial hypersensitivity to darkness. Furthermore, this phenotype was slightly accentuated in the *frs12-1*;amiR lines compared to *frs12-1* (Fig. 5C). These results confirm our observations at the gene regulation and protein interaction levels, indicating that FRS7 and FRS12 play coordinated functions in red light signaling to regulate light development.

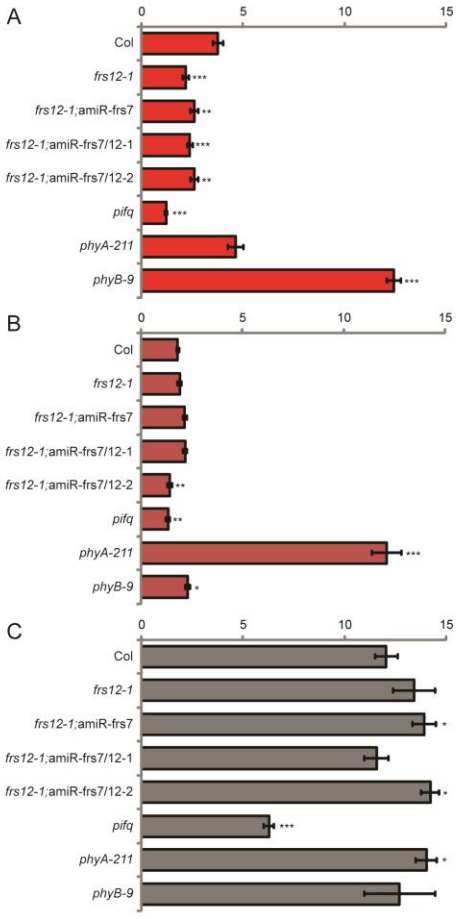

Figure 5. FRS7 and FRS12 Are Involved in the Regulation of Photo- and Skotomorphogenesis.
Hypocotyl elongation assays in continuous red **(A)**, far-red **(B)** and darkness **(C)** conditions. Stratified seeds of Col-0, *frs12-1*, *frs12-1*;amiFRS7, *frs12-1*;amiFRS7/12-1, *frs12-1*;amiFRS7/12-2, *pifq*, *phyA-211* and *phyB-9* were first exposed to continuous 80 µE of white light for 4 h at 21°C, then exposed to cD for 20 h. After this period seedlings were placed for 3 days at continuous monochromatic R (50 µE), FR (15 µE) light sources or at cD. Values represent the mean of 30 seedlings and error bars the SE. Asterisks indicate statistically significant differences between Col-0 wild-type *Arabidopsis* and the indicated genotypes (*p<0.05; **p<0.01; ***p<0.001, T-test).

In conclusion this work describes the characterization of FRS7 and FRS12, two proteins belonging to the family of the FRS transcription factors. The comprehensive systems biology approach employed here establishes the FRS7/FRS12 protein complex as a light regulated machinery involved in the control of multiple aspects of circadian rhythm, flowering time and red light signaling. Arabidopsis FRS7 and FRS12 loss-of-function lines show red light and dark hypersensitive phenotypes, suggesting the functions of these proteins in the modulation of light related development. At the protein level the functions of FRS7/FRS12 seem to be modulated by recruitment of the transcriptional co-repressor complex NINJA-TPL.

Genetic and molecular evidence suggest the functions of FRS7/FRS12 as red light regulators of light development, photoperiodism and flowering time (Fig. 6). We demonstrated the integration of FRS12 in a repressor complex composed of NINJA-TPL and FRS7 proteins. Linked to this observation, the night phased expression of *FRS7*, *FRS12* and their interacting partners further suggests the accumulation of the FRS7/FRS12 complex in darkness and its repression by light exposure. RNA-Seq analysis highlighted the function of the FRS7/FRS12 complex as a direct modulator of key regulators of light development such as PIL1, PIF4 and PRE1. PIFs accumulate in the darkness where they heterodimerize with HLH proteins such as PRE1 to induce etiolated growth (Leivar et al., 2009; Hao et al., 2012; Zhang et al., 2013). In the presence of light, the activity of PIFs is repressed by a bHLH/HLH complex composed of PIL1-HFR1 that promotes photomorphogenesis (Luo et al., 2014). The function of these regulators correlates with the observed hypocotyl elongation phenotypes in FRS7 and FRS12 loss-of-function lines showing partial red light and dark hypersensitivity. These phenotypes point to the involvement of FRS7/FRS12 in photo- and skotomorphogenesis and could be related to the miss-regulation of the activities of PIL1, PIF4 and PRE1. In this context, the FRS7/FRS12 complex could act as a red light-regulated mechanism balancing light development.

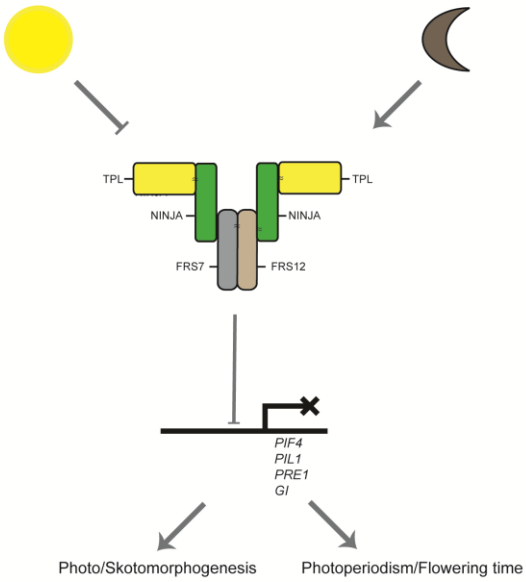

Figure 6. Proposed Model of Function of FRS7 and FRS12.
In darkness, FRS7 and FRS12 are produced whereas under (red) light exposure they are repressed. FRS7 and FRS12 integrate in a protein repressor complex with NINJA-TPL to bind and repress regulatory regions of genes involved in the modulation of photo/skotomorphogenesis, photoperiodism and flowering time (exemplified by *PIF4*, *PIL1*, *PRE1* and *GI*).

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4. Perspectives for future collaboration between units (1 page)

Over the years 2012 and 2013 Dr. Andrés Ritter benefited from a BELSPO post-doctoral fellowship to identify new molecular mechanisms of plant growth regulation linked to the Jasmonate plant hormone signalling pathway. This project was carried out in the laboratory of Prof. Alain Goossens at the department of Plant Systems Biology (PSB) located at Ghent University and led by Prof. Dirk Inzé.

The satisfactory progress of this project over these years allowed Dr. Andrés Ritter to characterize new transcriptional regulators of plant development that are controlled though signalling mechanisms integrating red light perception and jasmonate biosynthesis. The results presented here fully comply into the initial submitted BELSPO research project and respected the initially proposed research calendar. Moreover, this project established a new research line in the host lab that relies in the cross-talking relations between JA and light signalling. In this context, the laboratory of Prof. Goossens will continue exploring the functions of the FRS7 and FRS12 transcription factors through a systems biology strategy that will include e.g. Chromatine Immuno Precipitation – sequencing or additional TAP experiments. At his return to the Pontificia Universidad Católica Chile (PUC), Dr. Andrés Ritter will continue participating actively to this research project by guiding students and writing articles (see here below).

The future research line of Dr. Andrés Ritter at the PUC aims to unravel the functions of oxylipins in the brown algal model *Ectocarpus siliculosus* by the integration of forward and reverse genetics approaches. In this project Dr. Ritter will develop experiments in collaboration with Prof. Alain Goossens at the PSB department of Ghent University. He will benefit from the existing expertise at PSB to carry out a Yeast One-hybrid screen to identify algal transcription factors that can interact with regulatory DNA elements placed upstream of genes regulating algal defence. Pending on the success of this approach further characterizing experiments might be carried out at PSB, including e.g. transient activation assays in tobacco protoplasts or transcription factor hybridization to DNA protein-binding microarray containing all 11-mer DNA sequence variants (collaboration with Dr. Roberto Solano at the CNB-Spain).

5. Valorisation/Diffusion (including Publications, Conferences, Seminars, Missions abroad...

Over the course of his stay Dr. Andrés Ritter presented his results in prestigious conferences. Preliminary results of the results of this project were presented as a poster at the Nature conference entitled *Frontiers in plant biology: From discovery to applications* that was held in October 2012 in Ghent, Belgium. Moreover finalized results were recently selected to be exposed in an oral presentation at the *International Conference of Arabidopsis Research* that was held in July 2014 in Vancouver, Canada.

The achievements of this project will be further valorised in two peer review articles that will be shortly submitted to the prestigious journals E-Life and Molecular Plant.

6. Skills/Added value transferred to home institution abroad (1/2 page)

During his stay at the PSB department of Ghent University Dr. Andrés Ritter acquired extensive expertise on the molecular study of plant phytohormonal pathways. He learned state of the art techniques in the fields of transcriptomics, protein-protein and protein-DNA interactions. At his return Dr. Ritter will reintegrate his home laboratory at the department of ecology of the Pontificia Universidad Católica de Chile. His expertise and the collaborative links made over his stay in the PSB department of Ghent University will benefit his home institution and will greatly increase the chances of Dr. Ritter to obtain a future tenure track position as an associated professor. He plans to apply his expertise to a new research line that will search for oxylipin-related molecular pathways activating seaweed defence in the context of algae-herbivore interactions. In this context this project will integrate forward and reverse genetics approaches which Dr. Andrés Ritter learned over his stay at the PSB department. This ambitious project expects to describe original phytohormonal pathways in algae that will uncover new insights in the evolution of signalling mechanisms in eukaryotes.