Supporting information

Translational profile of developing phellem cells in *Arabidopsis thaliana* roots

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Table S1 List of primers used in overlapping PCR and in RT-qPCR.

Table S2 List of differentially expressed genes with adjusted p-value < 0.01 and |FC| > 2 of phellem and total fraction libraries.

Table S3 Functional enrichment analysis of DEGs found between GPAT5 (cluster 1) and UBQ10 (cluster 2) – associated translomes, performed using ClueGO. Gene Ontology terms with significant enrichment (adjusted p-value < 0.05) are presented.

Table S4A Network analysis of genes with increased expression in phellem cells, based on protein-protein interactions available in STRING database.

Table S4B List of genes with increased expression in phellem cells displayed in Cluster 1, after STRING analysis.

Table S4C List of genes with increased expression in phellem cells displayed in Cluster 2, after STRING analysis.

Table S4D List of genes with increased expression in phellem cells displayed in Cluster 3, after STRING analysis.

Table S5 List of differentially expressed TF genes and TF families in phellem cells.

Movie S1 Digital cross sections obtained from pGPAT5::BLRP-FLAG-GFP-RPL18 confocal image represented in Fig. S2.

Movie S2 Digital cross sections obtained from pUBQ10::BLRP-FLAG-GFP-RPL18 confocal image represented in Fig. S2

Fig. S1 Development and suberization of root periderm in *Arabidopsis thaliana*. (a) Plastic embedded cross-sections of pFAR4::GUS, from roots collected between 6 and 10 DAG. Represented sections were always taken below the hypocotyl-root junction, in different roots. Three representative root replicates (Root 1-3), from independent assays for each time-point, are shown. Black squares indicate the stele boundary cells, from pericycle daughter cells to new phellem cells. Red squares indicate the endodermis cells. (b) Fluorol yellow staining of the suberin (yellow) in primary roots. (c) pGPAT5::mCITRINE-SYP122 expression (yellow) and propidium iodide staining (red) of primary roots. Images were taken in the periderm development region, below the root-hypocotyl junction, between 6 and 10 DAG. (d) GPAT5::BLRP-FLAG-GFP-RPL18 expression (green) in the phellem differentiation zone, between 6 and 10 DAG. Cell walls (grey) were visualized by calcofluor white and suberin (purple) using Nile red staining, after ClearSee treatment. Yellow arrows indicate pGPAT5::BLRP-FLAG-GFP-RPL18 expression in the digital x-section. The red dashed line indicates the place of the digital cross section. The 3D maximum projection figures and digital x-sections were obtained by confocal laser scanning with Z-stack images. (e) Number of circumference cells in the stele from 5 to 10 DAG. Cell number was counted in plastic-embedded pFAR4::GUS sections (representatives in Fig. 1a and S1a). Middle line of the boxplot is median for $n \geq 6$ plants. Ep: epidermis; Co: cortex; En: endodermis. Scale bars: 50 μ m. Images are representative of all analyzed samples.

Fig. S2 Expression profile of pGPAT5::BLRP-FLAG-GFP-RPL18 and pUBQ10::BLRP-FLAG-GFP-RPL18 at 8 DAG, below the hypocotyl-root junction. The GPAT5 and UBQ10 promoters were used to drive expression of BLRP-FLAG-GFP-RPL18 cassette in phellem cells and in the total root cells, respectively (in green). Cell walls (grey) are visualized by calcofluor white and suberin (purple) by Nile red staining, after ClearSee treatment. The 3D maximum projection figures and digital cross-section were obtained by confocal laser scanning with Z-stack images. The red dashed lines 1 and 2 indicate the place of the respective digital cross-section. The yellow arrows indicate the BLRP-FLAG-GFP-RPL18 expression, driven by GPAT5 and UBQ10 promoters, in the 3D maximum projection and respective digital section. All the digital sections generated from the top to bottom of the presented images are shown in Movie S1 and S2, respectively. The GPAT5 promoter expression is always detected in the stele border region, where new phellem cells are formed. Contrasting, pUBQ10::BLRP-FLAG-GFP-RPL18 is detected in all cell types, as inner cells of the stele and cortex cells. Ep: epidermis; Co: cortex; En: endodermis. Scale bar: 50µm.

Fig. S3 Validation of differential expression levels in phellem (pGPAT5-associated / pUBQ10-associated RNA) for 5 selected genes. Linear regression with the correlation coefficient (R^2). RNA used in RT-qPCR and in RNA-seq was obtained by TRAP.

Fig. S4 Activation of NF-YC12 gene during periderm development of *Arabidopsis thaliana* roots. Plastic embedded cross-sections of pNF-YC12::GUS, taken below the hypocotyl-root junction between 7 and 8 DAG. Four independent representative roots are shown. Scale bar: 50 μ m. NF-YC12 was previously detected to be expressed in primary developed root tips and in leaf cells (Siefers et al., 2009). In this work, when observations are targeted to the secondary developed root, we detected punctate expression of NF-YC12 in new phellem cells. NF-Y transcription factors (also known as CCAAT box binding factors -CBFs) are discussed to be important regulators of numerous plant developmental and stress-induced responses. NF-YCs are sequence-specific transcription factors with histone-like subunits and are described to form heterotrimeric complexes composed of single subunits from each of three protein families: NF-YA, NF-YB, and NF-YC (Laloum et al., 2013; Zhao et al., 2017).

(a)

(b)

(c)

Fig. S5 Detailed representation of the different clusters identified from network represented in Figure 5 for predicted associations between DEGs with increased expression in phellem cells.. Protein identifiers are attributed to each network node to cluster 1 (a), cluster 2 (b) and cluster 3 (c). Node size corresponds to the Log2FC of each gene and thickening of connecting edges represent the confidence value for the association. Circle nodes represent genes present in the nuclear genome, while hexagon and diamond shapes represent genes present in plastid and mitochondrion genomes, respectively. Fill colors represent functions or processes attributed to each gene product. Nodes with black border represent TFs.

Table S1 List of primers used in overlapping PCR and in RT-qPCR.

Primer name	Sequence (5'-3')	Description
B1-BLRP-FLAG-GFP_F	AAAAAGCAGGCTTAATGGGACTTAACGATATCTTCGAAGCTCAGAAGAT TGAATGGCATGGAGGTGATTATAAGGATGATGATGATAAGGGAGGTGT GAGCAAGGGCGAGG	Primers used for overlapping PCR. BLRP - Biotin Ligase Recognition Peptide; FLAG - DYKDDDDK-tag; GFP-Green Fluorescence Protein; RPL18 – Ribosomal Protein L18.
GFP-RPL18_R	GATCAATACCGGACATCTTGACAGCTCGTCC	
GFP-RPL18_F	GGACGAGCTGTACAAGATGTCCGGTATTGATC	
B2-RPL18_R	AGAAAGCTGGGTTTAAACCTTGAATCCACGACTCTTCC	
AtGPAT5_qPCR1_F	GGGTTTGAGTGCACCAACTT	Primers used for RT-qPCR. The corresponding gene identifiers are: <i>GPAT5</i> - AT3G11430; <i>ASFT</i> - AT5G41040; <i>ABCG6</i> - AT5G13580; <i>UBCE2</i> - AT5G25760; <i>CKA1</i> - AT3G48750; <i>CKA2</i> - AT3G50000.
AtGPAT5_qPCR1_R	GGAGACAAGGCTCGAAAGTG	
AtASFT_qPCR1_F	GGTCAAACCTGAATCCGAGA	
AtASFT_qPCR1_R	CTTGGACTGCTTCCTCGTTC	
AtABCG6_qPCR1_F	ATGAACCAACTTCGGGTCTG	
AtABCG6_qPCR1_R	CGGGACAAGAAGAGAAGACG	
AtUBCE2_qPCR1_F	CTTGGACGCTTCAGTCTGTG	
AtUBCE2_qPCR1_R	GGCGAGGCGTGTATACATT	
CDKA ₁ _qPCR1_F	ATTGCGTATTGCCACTCTCATAGG	
CDKA ₁ _qPCR1_R	TCCTGACAGGGATACCGAATGC	
CKA2_qPCR1_F	ACCACCATTAACTGCGTCAAC	
CKA2_qPCR1_R	GATCTTGGCGAGAGAATCGGTATC	

Table S2 to Table S5 are in supplemental Excel files.

Movie S1: Digital cross sections obtained from *pGPAT5::BLRP-FLAG-GFP-RPL18* confocal image represented in Fig. S2. Expression profile of *pGPAT5::BLRP-FLAG-GFP-RPL18* at 8 DAG, below the hypocotyl-root junction. The *GPAT5* promoter was used to drive expression of *BLRP-FLAG-GFP-RPL18* cassette in phellem cells (in green). Cell walls (grey) are visualized by calcofluor white and suberin (purple) by Nile red staining, after ClearSee treatment. The digital cross-sections were obtained by confocal laser scanning with Z-stack images. The digital cross sections are visualized from the top to the bottom of 3D maximum projection in Fig. S2.

Movie S2: Digital cross sections obtained from *pUBQ10::BLRP-FLAG-GFP-RPL18* confocal image represented in Fig. S2. Expression profile of *pUBQ10::BLRP-FLAG-GFP-RPL18* at 8 DAG, below the hypocotyl-root junction. The *UBQ10* promoter was used to drive expression of *BLRP-FLAG-GFP-RPL18* cassette in the total root cells (in green). Cell walls (grey) are visualized by calcofluor white and suberin (purple) by Nile red staining, after ClearSee treatment. The digital cross-sections were obtained by confocal laser scanning with Z-stack images. The digital cross sections are visualized from the top to the bottom of 3D maximum projection in Fig. S2.

References:

- Laloum T, De Mita S, Gamas P, Baudin M, Niebel A. 2013.** CCAAT-box binding transcription factors in plants: Y so many? *Trends in Plant Science* **18**: 157–166.
- Siefers N, Dang KK, Kumimoto RW, Bynum IV WE, Tayrose G, Holt BF. 2009.** Tissue-specific expression patterns of Arabidopsis NF-Y transcription factors suggest potential for extensive combinatorial complexity. *Plant Physiology* **149**: 625–641.
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