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The Arabidopsis translatome cell-specific mRNA atlas

Mining suberin and cutin lipid monomer biosynthesis genes as an example for data application

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Plants consist of distinct cell types distinguished by position, morphological features and metabolic activities. We recently developed a method to extract cell-type specific mRNA populations by immunopurification of ribosome-associated mRNAs. Microarray profiles of 21 cell-specific mRNA populations from seedling roots and shoots comprise the Arabidopsis Translatome dataset. This gene expression atlas provides a new tool for the study of cell-specific processes. Here we provide an example of how genes involved in a pathway limited to one or few cell-types can be further characterized and new candidate genes can be predicted. Cells of the root endodermis produce suberin as an inner barrier between the cortex and stele, whereas the shoot epidermal cells form cutin as a barrier to the external environment. Both polymers consist of fatty acid derivates, and share biosynthetic origins. We use the Arabidopsis Translatome dataset to demonstrate the significant cell-specific expression patterns of genes involved in those biosynthetic processes and suggest new candidate genes in the biosynthesis of suberin and cutin.

Key words: cell-type specific expression, polysome immunopurification, translatome, suberin, cutin, endodermis, epidermis, arabidopsis

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Introduction

Plants consist of numerous specialized cell types with defined functions. For example, leaf mesophyll cells perform photosynthesis, whereas phloem cells transport sugars. Some cell types establish barriers within an organ or to the external environment, such as the root endodermis and leaf epidermis, respectively. To obtain insight into the genes involved in cell-specific specialization and function, plant scientists have labored to isolate specific cell-types to observe the regulated expression of cell-specific mRNAs. The methods that enable this are based on mechanical separation (i.e., epidermal peels,[1] phloem isolation[2]), laser microdissection of organs[3-5] or fluorescence-activated sorting of GFP-marked protoplasts.[6-10] However, these methods may not enable monitoring of rapid responses to chemicals or environmental cues. To successfully profile dynamic responses to hypoxia, we developed a non-invasive method to immunopurify polysome-bound mRNAs from specific cells by expressing a FLAG-tagged ribosomal protein under control of cell- and region-specific promoters.[11] This technique uses flash-frozen material to isolate the subset of cellular mRNAs associated with polysomes, the majority of which are likely to be undergoing translation. The profile of the ribosome-associate mRNAs or "translatome" is distinct from that of the transcriptome,[12,13] because mRNA abundance and translation are regulated by distinct processes. Hence, translatome profiling can provide a more precise readout of gene expression.

The Arabidopsis Translatome atlas can be used to study specific processes in one of the targeted cell types of control grown and hypoxia-treated seedlings. The data are accessible for individual genes via an eFP (electronic fluorescent pictograph) platform (efp.ucr.edu/).[11,14] This atlas of translatome profiles can be readily extended to the analysis of other

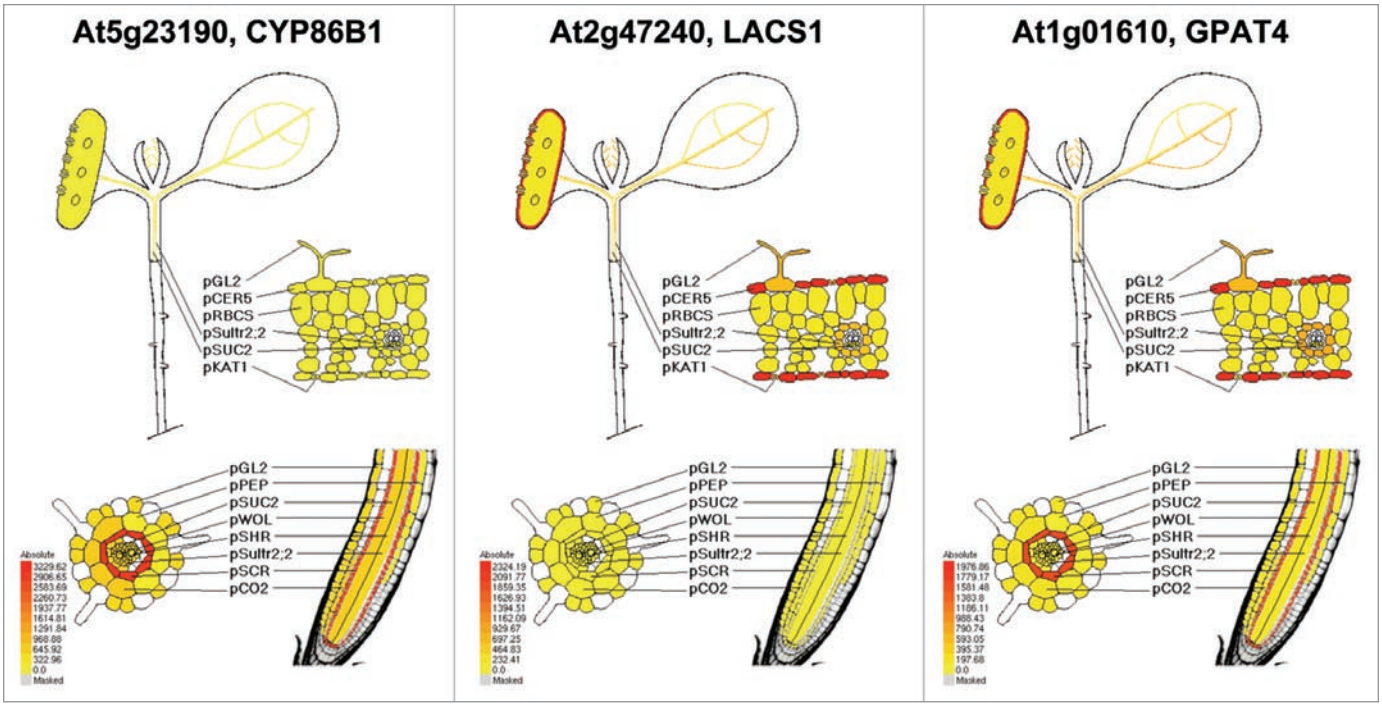

Figure 1. Three genes involved in suberin and cutin biosynthesis and their mRNA in translatomes of different cell populations of Arabidopsis, visualized via the eFP platform (efp.ucr.edu/). Absolute signal values of transcripts in translatomes isolated from cell populations.

transformable organisms or additional Arabidopsis cell types, such as the root pericycle and epidermis, cell types of the developing embryo, root, shoot and floral meristems and pollen. We demonstrate here how the Arabidopsis Translatome dataset can be used to study genes involved in the synthesis of lipid monomers that are precursors for the formation of the polymers suberin and cutin by the root endodermis and leaf epidermis, respectively.

Recent Advances in Suberin and Cutin Biosynthesis

Suberin and cutin are extracellular lipid polymers that provide barriers against water, ions and gases.[15,16] While cutin is present in the outer layers of the leaves, suberin is present at the outside of the roots (rhizodermis), where it influences water uptake and provides protection from pathogen and insect attack. Suberin also forms within organs, such as the border between bundle sheath cells and vasculature in C4 leaves and the endodermis in roots, controlling water and ion flux between the cortex and the vasculature.

The suberin polymer is composed of oxygenated fatty acid derivatives, alcohols and unsubstituted fatty acids, as well as glycerol and ferulic acid.[15] Suberin barriers differ in composition due to intricacies in the biosynthetic pathway that are not yet known in detail.[17] Cutin contains similar monomers, but also displays variations in composition.[16] Cuticular waxes are a third group of lipid polymers, produced in part by the monomer biosynthesis pathways common to cutin and suberin. Recent progress has been made in elucidation of single steps involved in oxygenated fatty acid synthesis, elongation and modification through recognition of the participant enzymes. A number of genes have been associated with suberin and/or cutin biosynthesis by use of Arabidopsis mutants. Here, we use the Arabidopsis Translatome dataset to confirm the localized expression of genes involved in suberin and cutin biosynthesis and identify genes that are likely to be involved in lipid monomer biosynthesis associated with production of these lipid biopolymers in the endodermis or epidermis of Arabidopsis.

The Arabidopsis Translatome dataset consists of 21 mRNA profiles including root and root tip endodermis (pSCR) and shoot epidermis (pCER5) mRNA populations. The dataset includes two readouts: (1) the absolute signal value for a given mRNA, or mRNA abundance; (2) the fold enrichment of an mRNA in a cell type/population relative to non-overlapping cell types or cell populations evaluated for the same organ. The data can be displayed for individual genes, as shown for three endodermal and shoot epidermal-enriched mRNAs (**Fig. 1**). The mRNAs enriched in specific cell types are likely to be involved in cell specific processes. By use of GO term annotations, we recognized that mRNAs involved in suberin or cutin biosynthesis were significantly overrepresented in the endodermis (pSCR, i.e., peroxidase activity, adj. p-value 9.76E-09; lipid binding, 6.89E-03; fatty acid metabolic process, 1.47E-09; lipid transport, 3.17E-03; acetyl-CoA biosynthetic process from pyruvate, 3.68E-03) and the shoot epidermis (pCER5, i.e., carboxylesterase activity, 3.82E-09; fatty acid (omega-1)-hydroxylase activity, 7.15E-03; lipid metabolic process, 4.60E-09; cuticle development, 2.91E-05; cellulose and pectin-containing cell wall modification, 6.08E-03) (reviewed in ref. 11). This motivated us to further explore the cell-specific expression of known genes of suberin biosynthesis.

One major reaction in early suberin and cutin biosynthesis is the fatty acid elongation via a multi-enzyme complex (FAE).[15] The rate-limiting step of this enzyme complex is the β-ketoacyl-CoA synthase (KCS), encoded by a family of 21 genes in Arabidopsis. Of those, six are involved in suberin and cutin biosynthesis (*KCS1*,[18] *KCS2*,[19,20] *KCS6* (*CUT1*),[21] *KCS10* (*FDH*),[22] *KCS18* (*FAE1*),[23] *KCS20*,[20]). Of the remaining *KCS* genes, several show highly specific expression in either the root endodermis or the leaf epidermis, and are therefore potential targets for future studies (**Table 1**). Another enzyme of this complex is the β-ketoacyl-CoA reductase (KCR), encoded by two genes in Arabidopsis. Whereas *KCR1* is essential for suberin and wax biosynthesis[24] and is enriched in the root endodermis (**Table 1**), *KCR2* is neither essential nor found to be expressed in our dataset.

Following the elongation step, the fatty acids are hydroxylated at the ω-position by NADPH-dependent cytochrome P450 monooxygenases of the CYP86 and CYP94 families.[15] Six of the 11 putative *CYP86* genes were found to modify suberin or cutin composition when knocked out in Arabidopsis, of which all have mRNAs specifically enriched in either the endodermis or epidermis in the Translatome atlas (*CYP86A1,*[25,26] *CYP86A2,*[27] *CYP86A4,*[28] *CYP86A8,*[29] *CYP86B1*/*CYP86B2,*[30,31]). These data affirm the cell-specific importance of *CYP450* genes. The *CYP94* gene family has only one out of six members, which is specific for the leaf epidermis, but has not yet been characterized in respect to fatty acid hydroxylation. Two additional CYP450 genes, *CYP705A1* (At4g15330) and *CYP714A2* (At5g24900) are enriched in the endodermal mRNA population, and could be also involved in suberin biosynthesis, while eight additional *CYP450* genes are specifically enriched in the leaf epidermis (**Table 1**). One of those, *CYP77A6* was recently found to be involved in cutin biosynthesis.[28]

An alternative pathway to modify very long chain fatty acids involves alcohol (ω-hydroxyacid) dehydrogenases and aldehyde (ω-oxo-acid) dehydrogenases.[30] The first enzyme of this pathway was characterized in Arabidopsis,[32] but the second is unknown. Our data suggest a potential candidate for this enzymatic step (**Table 1**).

Suberin and cutin are oligomers of fatty acids, esterified with either glycerol or ferulic acid. This oligomer formation requires the activity of acyl transferases (ACTs), such as acyl-CoA:glycerol-3-phosphate acyltransferase (GPAT) and aliphatic suberin feruloyl transferase (ASFT). Arabidopsis contains nine putative GAPT and two putative ASFT genes. Of the *GAPT*s, four are involved in suberin and cutin synthesis (*GAPT4,*[25] *GAPT5,*[33] *GAPT6,*[28] *GAPT8,*[25]). *GAPT3* is also enriched among shoot epidermis mRNAs and could therefore be involved in cutin biosynthesis (**Table 1**). The recently described *ASFT* is involved in modification of suberin composition,[31,34] and is specifically expressed in root endodermis (**Table 1**). A second *ASFT* candidate, At5g63560, is also slightly enriched in this cell type.[11]

Outlook

In addition to biosynthetic pathways, several other processes are required for the formation and deposition of suberin, including fatty acyl or wax exporters (ABC transporters, i.e., CER5, WBC11), extracellular lipid transfer proteins, and signaling components (i.e., WIN1). With the available dataset, confirmed and potential new candidates can be selected for further in-depth studies. The 260 endodermis-specific and the 511 epidermis-specific genes identified in the Translatome atlas provide a large resource for the further exploration of genes associated with suberin and cutin synthesis, transport and deposition.[11] Of the 15 genes highly enriched in both mRNA populations, only 11 have assigned functions. The epidermal data is also likely to yield leads in wax biosynthesis. Furthermore, the 26 and 24 transcription factors enriched in the root endodermis (pSCR) and shoot epidermis (pCER5), respectively, deserve examination in experiments that couple genetic, biochemical and enzymatic approaches. In conclusion, the highly cell-specific biosynthesis of lipid polymers provides one example of the data potential of the Arabidopsis Translatome atlas.

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Table 1. Summary of selected gene families associated with suberin and cutin biosynthesis—known members and potential new candidates

AGI ID	Gene name	Described function	Cell type enriched[11]	SLR R SCR (endodermis)	SLR S CER (epidermis)
3-ketoacyl CoA synthase					
At1g01120	*KCS1*	cutin[18]	R endo/S epi	2.73	2.32
At1g04220	*KCS2 (DAISY)*	suberin,[19] cutin[20]	n.s.	0.97	0.88
At1g07720	*KCS3*	potential new target	S epi	0.06	2.68
At1g25450	*KCS5*	potential new target	S epi	-0.16	2.47
At1g68530	*KCS6 (CUT1, CER6)*	cutin[21]	n.s.	0.07	1.75
At2g16280	*KCS9*	potential new target	S epi	0.85	2.46
At2g26250	*KCS10 (FDH)*	cutin[22]	R endo	2.29	2.27
At2g46720	*KCS13 (HIC)*	potential new target	S epi	0.03	1.79
At4g34250	*KCS16*	potential new target	S epi	0.27	2.15
At4g34510	*KCS17*	potential new target	R endo	1.68	0.08
At5g04530	*KCS19*	potential new target	S epi	0.04	2.98
At5g43760	*KCS20*	cutin[20]	n.s.	1.19	1.93
ketoacyl CoA reductase					
At1g67730	*KCR1*	cutin, suberin[24]	R endo	1.71	1.58
long-chain acyl-CoA synthetases					
At2g47240	*LACS1 (CER8)*	cutin[35]	S epi	0.11	3.36
At1g49430	*LACS2*	cutin[36]	R endo	2.34	2.02
At1g64400	*LACS3*	potential new target	R endo	1.55	1.81
fatty acid omega-hydroxylase					
At5g58860	*CYP86A1 (HORST)*	suberin[26]	R endo	2.33	-0.01
At4g00360	*CYP86A2 (ATT1)*	cutin[27]	R endo/S epi	2.68	2.62
At1g01600	*CYP86A4*	cutin[28]	S epi	1.53	2.37
At2g45970	*CYP86A8 (LCR)*	cutin[29]	R endo/S epi	1.97	2.65
At5g23190	*CYP86B1*	suberin[30]	R endo	2.71	-0.02
At5g08250	*CYP86B2*	suberin[30]	R endo	1.90	0.02
At5g63450	*CYP94B1*	potential new target	S epi	0.79	2.04
other CYP450 enzymes					
At4g15330	*CYP705A1*	potential new target	R endo	1.94	0.34
At4g22710	*CYP706A2*	potential new target	S epi	-0.52	2.10
At2g34490	*CYP710A2*	potential new target	S epi	0.15	2.52
At5g24900	*CYP714A2*	potential new target	R endo	1.24	-0.12
At3g53280	*CYP71B5*	potential new target	S epi	0.08	2.24
At2g26710	*CYP734A1*	potential new target	S epi	-0.19	2.54
At3g10570	*CYP77A6*	cutin[28]	S epi	0.07	1.82
At1g11600	*CYP77B1*	potential new target	S epi	-0.02	1.92
At5g09970	*CYP78A7*	potential new target	S epi	0.13	1.31
At5g52320	*CYP96A4*	potential new target	S epi	-0.33	2.11
alcohol dehydrogenase					
At1g72970	*HTH*	cutin[32]	S epi	0.37	2.64
aldehyde dehydrogenase					
At4g36250	*ALDH*	potential new target	S epi	-0.15	2.47
glycerol-acyl-transferase					
At4g01950	*GPAT3*	potential new target	S epi	0.32	2.12
At1g01610	*GPAT4*	cutin[25]	R endo	3.48	2.88

SLR, mean signal log ratio of pairwise comparisons of a specific cell type (here: pSCR or pCER5) to all other non-overlapping cell types of the same organ;[11] n.s., not significant.

Table 1. Summary of selected gene families associated with suberin and cutin biosynthesis—known members and potential new candidates

At3g11430	*GPAT5*	suberin[33]	R endo	2.80	-0.18
At2g38110	*GPAT6*	cutin[28]	R endo	3.01	0.41
At4g00400	*GPAT8*	cutin[25]	R endo/S epi	2.37	2.83
		feruloyl-acyl-transferase			
At5g41040	*ASFT (ACT)*	suberin[31, 34]	R endo	3.43	0.14

SLR, mean signal log ratio of pairwise comparisons of a specific cell type (here: pSCR or pCER5) to all other non-overlapping cell types of the same organ;[11] n.s., not significant.

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