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Cell-specific protein profiling in *Arabidopsis thaliana* trichomes: identification of trichome-located proteins involved in sulfur metabolism and detoxification

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Abstract

Metabolite, protein, and transcript analysis at the cellular level gives unparalleled insight into the complex roles tissues play in the plant system. However, while capillary electrophoresis and PCR amplification strategies make the profiling of metabolites and transcripts in specific cell types possible, the profiling of proteins in small samples represents a bottleneck. Here for the first time protein profiling has been achieved in a specific plant cell type: The application of specific cell sampling and shotgun peptide sequencing (nano LC/MS/MS) resulted in the identification of 63 unique proteins from pooled *Arabidopsis* trichome cells. A complete *S*-adenosylmethionine pathway cluster, two *S*-adenosylmethionine synthase isoforms, a glutathione S-conjugate translocator and other proteins involved in sulfur metabolism and detoxification are shown to be present in these cells, in agreement with previous work done at the level of trichome transcript analysis. The technology described here brings the simultaneous identification and localization of physiologically relevant cellular proteins within reach.

Keywords: Plant shotgun proteomics; Nano-LC/MS; Sulfur metabolism; Single cells

1. Introduction

High spatial and temporal resolution of transcript and metabolite profiling at the single-cell level requires extremely sensitive analytical methods: capillary electrophoresis and PCR amplification strategies combined with specific sampling methods have been used successfully at this level (Fricke et al., 1994; Brandt et al., 1999, 2002; Tomos and Leigh, 1999; Koroleva et al., 2000; Kehr, 2001; Lochmann et al., 2001; Nakazono et al., 2003). Initial studies on single cell proteins using two-dimensional gel electrophoresis (2DE) in combination with native gels and mass spectrometry allowed the analysis of target proteins from guard cells (Li and Assmann, 2000). However, until now large-scale identification of proteins on the cell-specific level of plants failed due to small sample volumes, low protein levels, and complex tissue structure (Kehr, 2001, 2003). The use of oligonucleotide- and cDNA-based microarrays is providing unprecedented insights into the regulation of global patterns of gene expression (Schulze and Downward, 2001). Nevertheless, since protein abundance does not always correlate with transcript levels (Gygi et al., 1999a; Greenbaum et al., 2003) and since the cellular localization and turnover rate of biologically active protein can only be determined directly, the development of sensitive methods for comprehensive analysis of specific cell type protein expression patterns in *Arabidopsis* and other plants is needed. The combination of HPLC separation of complex peptide mixtures with subsequent tandem mass spectrometry (LC/MS/MS) provides an alternative tool for proteome and subproteome analysis (Link et al., 1999; Yates et al., 1999;

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Washburn et al., 2001; Wolters et al., 2001; McDonald and Yates, 2002; VerBerkmoes et al., 2002; Yi et al., 2002; Weckwerth et al., 2004; Wienkoop and Saalbach, 2003). The advantages of this technology are the capacity for high throughput, no bias against protein classes (as in the case using 2DE (Washburn et al., 2001)), and small sample volumes combined with high detection sensitivity. A detailed comparison of 2DE and shotgun proteomics is found in an excellent study by Koller et al. (2002). In the case of *Arabidopsis*, sufficient genomic sequence information is available and identification of proteins is straightforward (Anon, 2000; Schoof et al., 2002; Weckwerth et al., 2004).

Trichomes provide a worthy model system for cell-specific analyses since they are located on the surface of the leaves, thus enabling easy access and fast sample collection. In *Arabidopsis*, trichomes are single-cell hairs derived from single epidermal cells that differentiate to form a characteristic three-branched structure (Hülskamp et al., 1994; Larkin et al., 2003). The physiological function of trichomes is still highly debated. It has been suggested that they are involved in protecting the plant against diverse environmental challenges, including mechanical injury, pathogen attack, and damage from a wide range of herbivores (Gutierrez-Alcala et al., 2000). In addition, tissue injury triggers specific defence mechanisms in which trichomes might be involved. In *Arabidopsis*, trichomes are single-cells with a wide variety of suggested functions. However, it is not known whether trichomes produce molecules toxic to pests or merely act as a simple physical barrier to predators. Kelsey (1984) reported that glandular trichomes are responsible for the synthesis and secretion of a broad range of natural products such as terpenoids, cannabinoids, sucrose esters, and phenolic compounds, many of which are known to be involved in plant defence mechanisms (Gershenzon et al., 1992; McCaskill et al., 1992). Further evidence suggests that trichomes participate in heavy metal detoxification and other stress conditions (Salt et al., 1995; Garcia-Hernandez et al., 1998).

Recently, one- or multidimensional LC/MS/MS technology has emerged as a tool enabling the analysis of crude complex protein mixtures (Washburn et al., 2001; Wolters et al., 2001; Yi et al., 2002). A new challenge is the analysis of the proteome of specific cell types, starting with an extremely small amount of total protein. This kind of analysis is combining rapid identification of proteins with their cellular localization, thereby assigning genes to specific cell types. Here we describe a convenient approach to characterizing a cell protein profile from *Arabidopsis* trichomes using 1-D nano LC/MS/MS. Several proteins involved in sulfur metabolism are part of the *Arabidopsis* trichome protein profile. Interestingly, a glutathione S-conjugate ABC transporter (At1g30400, AtMRP1) was identified, supporting the function of trichomes as a detoxification system according to previous studies (Gutierrez-Alcala et al., 2000).

2. Experimental procedures

2.1. Sampling of single trichomes of Arabidopsis leaves

Before the ablation of trichomes, *Arabidopsis* (ecotype columbia 0) leaves were briefly frozen in liquid nitrogen. Single trichomes were clipped off using forceps and inspected with a binocular microscope to confirm sample purity. Trichomes that exhibited remainders of neighbouring cells were discarded. The cells were collected in 5 μl of trypsin buffer (5% ACN/100 mM NH_4HCO_2/5 mM $CaCl_2$). Compared to animal cells such as mouse oocytes, which contain about 17 ng proteins per cell (Sasaki et al., 1999), plant cells are expected to have a considerably lower protein content (Kehr, 2001). The estimated protein amount was about 1 μg of the complex protein mixture per 1000 cells (about 1 ng per cell). Approximately 1000–2000 cells in total were collected, and between 1 and 2 μg protein was analysed per run.

2.2. Sampling of epidermal cell sap of Arabidopsis leaves

Epidermal cell sap was collected according to Brandt et al. (1999). Briefly, borosilicate glass capillaries (WPI, Berlin, Germany) were pulled on a List pipette puller (Darmstadt, Germany). Tip aperture was 1–10 μm. The capillaries were mounted on a micromanipulator and inserted into an epidermal leaf cell. Cell samples were collected under an Optiphot 2 microscope (Nikon, Duesseldorf, Germany). A humidifier was used to prevent desiccation of the sample in the capillary. Immediately after withdrawal of the capillary from the tissue, its content was released into a sterile, 0.5 ml reaction tube containing 5 μl of trypsin buffer (5% ACN/100 mM NH_4HCO_2/5 mM $CaCl_2$).

2.3. Protein digestion and nano LC/MS/MS analysis

The proteins were digested by adding 1 μl trypsin beads (Poroszyme^{TM} bulk immobilized enzyme, Applied Biosystems, Darmstadt). After centrifugation the beads and insoluble material were removed and the tryptic peptides were separated through a reversed-phase (RP) nano column (75 μm ID, 10 cm, 3 μm C18, 300 A, Optronis, Kehl) with an HPLC (Surveyor, Thermofinnigan, San Jose). The flow rate was split after the pumps. The tryptic digest was first loaded onto a peptide trap (Bischoff Chromatography, Leonberg) with a flow rate of 2 μl/min and subsequently washed for 5 min with 100% solvent A (0.1% formic acid in water). After

washing, elution of the peptides and gradient separation was done with a flow rate of 100–200 nl/min using different gradient durations for replicate analysis (40, 60, and 90 min with 0–100% solvent B (0.1% formic acid in acetonitrile)). The voltage was applied directly to the analyte solution using a T-piece. Eluted peptides were continuously analysed using an ion trap (LCQ Deca Xplus, Thermofinnigan) by selecting the three most abundant signals of a survey scan (mass range m/z 500–2000) for sequential MS/MS fragmentation. The combined MS/MS spectra from repetition runs of trichome samples were used to search the MIPS annotated *Arabidopsis thaliana* database (http://www.mips.biochem.mpg.de/) with the MS/MS ion search option of the Mascot search program (www.matrixscience.com), which calculates the predicted fragment ion masses from each peptide of a database sequence and scores it in comparison to the observed ion masses. Additionally, Bioworks 3.1 and SEQUEST (Thermofinnigan) was used. The SEQUEST algorithm correlates experimentally obtained CID spectra to the theoretical CID spectra of peptide sequences from a specified database and assigns a cross-correlation (Xcorr) score (Eng et al., 1994). The following criteria were applied for protein identification:

(i) A protein hit was only accepted if found in at least two replicate runs of the samples.
(ii) For the SEQUEST search we used stringent criteria (Xcorr $1^* = 2$; $2^* = 1.5$; $3^* = 3.3$, $\Delta Cn > 0.1$, only fully tryptic ends) for positive identification proven to provide the lowest false positive rates (Peng et al., 2003).
(iii) Only hits with at least one peptide in the first rank and significant probability based scores >49 were accepted from Mascot search (Perkins et al., 1999; Santoni et al., 2003).
(iv) Comparison of Mascot and SEQUEST results.

3. Results and discussion

3.1. Cell sampling and nano LC/MS/MS

Since accurate cell sampling is crucial to sample purity, single cells were harvested and inspected using a binocular microscope (see Section 2), ensuring that cross contamination from other compartments was prevented to the greatest possible extent. The protein content achievable with this method was about 1 μg total protein per 1000 cells. The resulting complex protein mixture was sampled directly into trypsin buffer (see Section 2) allowing maximum sample recovery.

Subsequent to tryptic digestion, peptides of the complex protein mixture were directly loaded on RP chromatography (nano LC) without further purification. Nano LC delivers much lower flow rates, down to 100–200 nl/min, and enables the use of 75–100 μm ID RP-columns compatible with very small sample volumes and protein amounts. A comparison of capillary LC (180–200 μm ID columns) with flow rates of 1–2 μl/min showed that nano LC/MS/MS and flow rates of about 100–150 nl/min enhances the sensitivity and quality of MS/MS spectra, especially for low abundance complex peptide mixtures which is in agreement with work done by others (Emmett and Caprioli, 1994; Martin et al., 2000).

3.2. Sulfur metabolism in trichomes

Sulfur plays an important role in the regulation of plant growth and development. Sulfur metabolism was previously implicated in trichome function when high levels of *O*-acetylserine(thio)lyase, serine acetyltransferase, γ-glutamylcysteine synthase and glutathione synthethase transcripts were found in leaf trichomes of *Arabidopsis* (Gotor et al., 1997; Gutierrez-Alcala et al., 2000).

In this study, several proteins involved in sulfur metabolism were identified in trichome extracts (Table 1, Fig. 2). Our analysis revealed the presence of *S*-adenosylmethionine synthase (AdoMet synthase) (Table 1 and Fig. 1) and methionine synthase (Table 1). The good quality of the spectra and the number of peptides per protein gives a quantitative estimate of high abundance (Florens et al., 2002; McDonald and Yates, 2002; Tabb et al., 2002). Interestingly, two forms of *S*-adenosylmethionione synthase, At4g018501 and At3g17390 (Table 1), were identified with slight differences in their amino acid sequences, which resulted in significant peptide mass differences. This result indicates that two isoforms of AdoMet synthase may exist in trichomes. The simultaneous existence of different isoforms is consistent with previous observations of two AdoMet synthase isoforms in *Arabidopsis* seeds (not necessarily within the same cell type) at the time of radicle protrusion (Gallardo et al., 2002). The sequences reported for AdoMet synthase (accession 9229983) and AdoMet synthase 2 (accession 127045) are identical to those of At4g018501 and At3g17390, respectively, described here.

AdoMet synthase catalyses the transfer of the adenosyl group from ATP to the sulfur atom of methionine. S-adenosylmethionine participates in the methylation of nucleic acids, proteins, carbohydrates, membrane lipids, and the cell wall (lignification) and thus, is a universal reagent for methylation in the cell. The active methyl group can be transferred to other acceptors via methyl transferases.

Another enzyme that we identified in trichome extracts participates in C1 metabolism: serine hydroxymethyltransferase (SHMT; Table 1) catalyses the conversion of serine into glycine, which is subsequently oxidized by glycine decarboxylases (Mouillon et al., 1999).

Table 1
List of identified trichome proteins

Accession no.	Protein [matched peptides (Mascot)]	Found with	Protein score (Mascot)	Xcorr (Sequest) [charge state]
At5g17920	5-Methyltetrahydropteroyltriglutamate-homocysteine *S*-methyltransferase (methionine synthase) [7]	Mascot/Sequest	239	3.78 [3+]
At3g52930	Fructose bisphosphate aldolase-like protein [5]	Mascot/Sequest	155	3.54 [2+]
At4g13930	Serine hydroxymethyltransferase [4]	Mascot/Sequest	142	2.94 [2+]
At5g02500	dnaK-type molecular chaperone hsc70.1 [4]	Mascot/Sequest	127	3.26 [2+]
At3g17390	*S*-adenosylmethionine synthetase [4]	Mascot/Sequest	106	1.55 [2+]
At4g01850	*S*-adenosylmethionine synthase 2 [5]	Mascot/Sequest	102	3.6 [2+]
At2g21660	Glycine-rich RNA binding protein [3]	Mascot/Sequest	98	4.18 [2+]
At1g49240	Actin8 [4]	Mascot/Sequest	95	2.54 [2+]
At3g59010	Pectinesterase precursor-like protein [4]	Mascot/Sequest	94	3.08 [2+]
At5g65690	Phosphoenolpyruvate carboxykinase (ATP) [6]	Mascot/Sequest	94	1.94 [2+]
At5g03300	Adenosine kinase (MOK16.21) [5]	Mascot/Sequest	90	3.82 [2+]
At5g02870	60S ribosomal protein-like [3]	Mascot/Sequest	82	3.54 [2+]
At1g55490	Rubisco subunit binding-protein beta subunit [6]	Mascot/Sequest	82	2.59 [2+]
At3g05020	Acyl carrier protein 1 precursor (ACP) [2]	Mascot/Sequest	80	4 [2+]
At1g56075	elongation factor, putative [5]	Mascot/Sequest	78	4.24 [2+]
At1g26630	Initiation factor 5A-4, putative [3]	Mascot/Sequest	76	1.73 [2+]
At2g36530	Enolase (2-phospho-D-glycerate hydroylase) [4]	Mascot/Sequest	75	3.25 [2+]
At5g10360	40S ribosomal protein S6 [3]	Mascot/Sequest	71	2.38 [2+]
At4g02660	Unknown protein [14]	Mascot/Sequest	70	1.97 [2+][a]
At1g79550	Putative phosphoglycerate kinase [4]	Mascot/Sequest	65	2.03 [2+]
At3g44320	Nitrilase 3 [3]	Mascot/Sequest	62	2.89 [2+]
At5g10450	14-3-3-like protein AFT1 [2]	Mascot/Sequest	59	1.53 [2+]
At5g52310	Low-temperature induced protein 78 (sp|Q06738) [6]	Mascot/Sequest	58	1.96 [2+]
At3g55440	Cytosolic triosephosphatisomerase [3]	Mascot/Sequest	56	2.69 [2+]
At5g40370	Glutaredoxin -like protein [2]	Mascot/Sequest	55	1.92 [2+]
At4g09320	Nucleoside-diphosphate kinase (EC 2.7.4.6) [1]	Mascot/Sequest	50	3.01 [2+]
At1g55860	Ubiquitin-protein ligase 1, putative [13]	Mascot	111	
At1g30400	Glutathione S-conjugate ABC transporter (AtMRP1) [8]	Mascot	88	
At5g24740	VPS13-like protein [14]	Mascot	81	
At1g21210	Unknown protein [8] wall-associated kinase 4	Mascot	80	
At3g48190	Ataxia-telangiectasia mutated protein AtATM [15]	Mascot	80	
At3g62900	Unknown protein [6]	Mascot	78	
At3g42670	Unknown protein [9]	Mascot	76	
At4g14970	Unknown protein [6]	Mascot	75	
At5g40820	AtRAD3 (dbj|BAA92828.1) [9]	Mascot	74	
At1g13210	Putative calcium-transporting ATPase [8]	Mascot	73	
At2g13330	Putative retro-element pol polyprotein [5]	Mascot	66	
At1g48090	Unknown protein [8]	Mascot	65	
At5g37500	Guard cell outward rectifying K+ channel [5]	Mascot	62	
At1g32530	Unknown protein [5]	Mascot	60	
At3g09790	Polyubiquitin [3]	Mascot	60	
At3g25450	Unknown protein [6]	Mascot	60	
At3g02260	Unknown protein [12]	Mascot	59	
At4g13940	Adenosyl homocysteine hydrolase [4]	Mascot	59	
At2g25730	Unknown protein [8]	Mascot	59	
At4g16890	Disease resistance RPP5 like protein [7]	Mascot	52	
At5g59980	Unknown protein [7]	Mascot	51	
At3g01320	Unknown protein [8]	Mascot	50	
At1g23410	Ubiquitin extension protein, putative	Sequest		4.15 [2+]
At1g20440	Hypothetical_protein	Sequest		3.61 [3+]
At2g42740	60S ribosomal protein L11B	Sequest		2.89 [2+]
At3g03250	Putative_UDP-glucose_pyrophosphorylase	Sequest		2.66 [2+]
At3g18930	Unknown protein	Sequest		2.35 [2+]
At1g11655	Unknown protein	Sequest		1.75 [2+]
At1g04820	Tubulin alpha-2alpha-4 chain	Sequest		1.74 [2+]
At3g19890	Hypothetical protein	Sequest		1.73 [2+]
At1g21810	Myosin-like protein	Sequest		1.73 [2+]
At5g38410	Ribulose bisphosphate carboxylase small chain 3b precursor	Sequest		1.71 [2+]
At3g51540	Putative protein	Sequest		1.65 [2+]
At4g04510	Putative receptor-like protein kinase	Sequest		1.57 [2+]

Table 1 (*continued*)

Accession no.	Protein [matched peptides (Mascot)]	Found with	Protein score (Mascot)	Xcorr (Sequest) [charge state]
At1g19840	Auxin-induced_protein,_putative	Sequest		1.57 [2+]
At4g13070	Putative protein	Sequest		1.54 [2+]
At3g03330	Unknown protein	Sequest		1.54 [2+]

[a] Potentially phosphorylated.

Serine hydroxymethyltransferase (SHMT) has not previously been described in trichomes. Recently, Mouillon et al. (1999) suggested that SHMT probably plays an important role in tissues with a high protein turnover: rapidly dividing tissues, those with a high protease activity, or tissues displaying autophagic processes. To what extent this occurs in trichome cells remains unclear. C1 metabolism is involved in a variety of cellular processes such as methyltransfer reactions, purine, thymidate, and methionine biosynthesis (Cossins and Chen, 1997). Since a *N*-methyltetrahydrofolate-dependent methionine synthase (Table 1, At5g17920.1) has been identified in this study, the association of serine hydroxymethyltransferase with methionine biosynthesis may close the circle between C1 and methionine metabolism (Fig. 2).

3.3. Glutaredoxin and glutathione S-conjugate translocator (AtMRP1)

The possibility that trichomes play a role in specific defence mechanisms such as oxidative stress response and detoxification has been discussed (Gutierrez-Alcala et al., 2000). Measuring the specific protein profile in *Arabidopsis* trichomes led to the identification and localization of proteins involved in these mechanisms such as glutaredoxin and a glutathione S-conjugate translocator (Table 1). Glutaredoxin is a 12-kDa thiotransferase that reduces disulfide bonds of other proteins and maintains the redox potential of cells (Lee et al., 2002). In addition to its oxidoreductase activity, Lee et al. (2002) reported that glutaredoxin from rice could also function as a glutathione-dependent peroxidase. Because of its antioxidant activity, glutaredoxin protects glutamine synthase from oxidative damage. In the presence of glutathione as an electron donor, glutaredoxin has been shown to reduce the disulfide bonds of other proteins such as dehydroascorbate or 2-hydroxyethylene disulfide. Thus, the function of glutaredoxin is correlated with glutathione. Recently, glutathione biosynthesis was observed in *Arabidopsis* trichomes (Gutierrez-Alcala et al., 2000), corroborating the localization of glutaredoxin within trichomes. Furthermore, the presence of glutaredoxin seems to emphasize the function of glutathione as an antioxidant in stress responses of trichomes. A well-known detoxification mechanism in plants is the glutathione *S*-transferase-catalysed glutathionation of toxins. The resulting

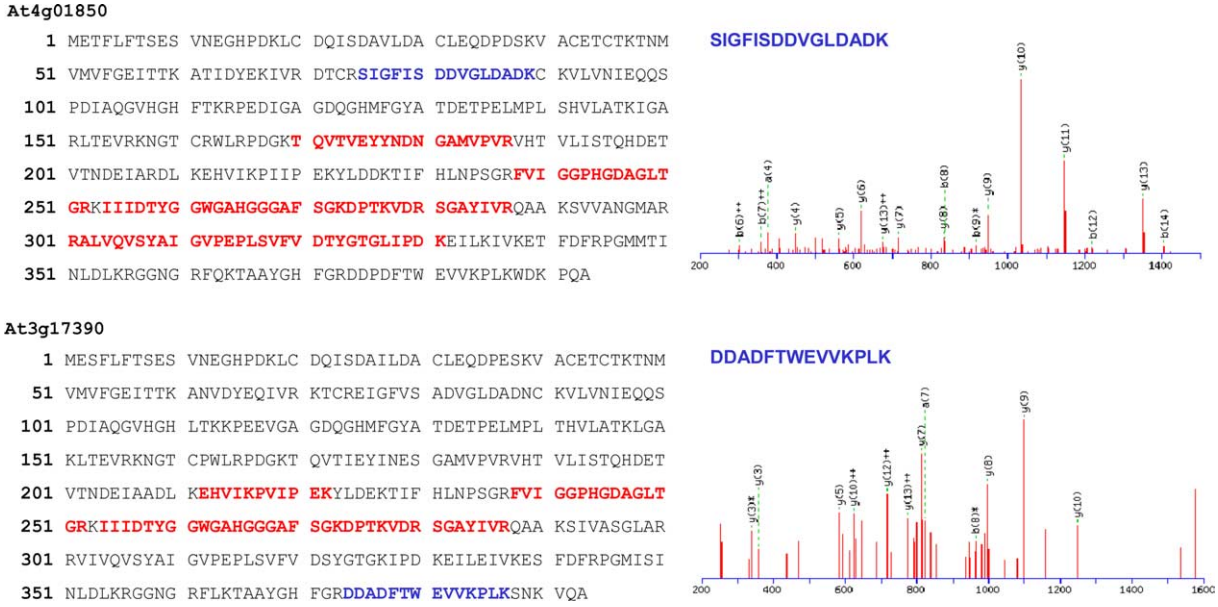

Fig. 1. Discrimination of isoenzymes using shotgun proteomics. The two isoforms of *S*-adenosylmethionine synthase At4g01850 and At3g17390 are distinguished by the detection of specific peptide masses and the corresponding MS/MS fragmentation. Significant peptide hits are highlighted in red, MS/MS fragmentation is shown for blue highlighted peptides different in the corresponding isoform. At3g17390 is specifically distinguished by the peptides DDADFTWEVVKPLK and EHVIKPVIPEK among others.

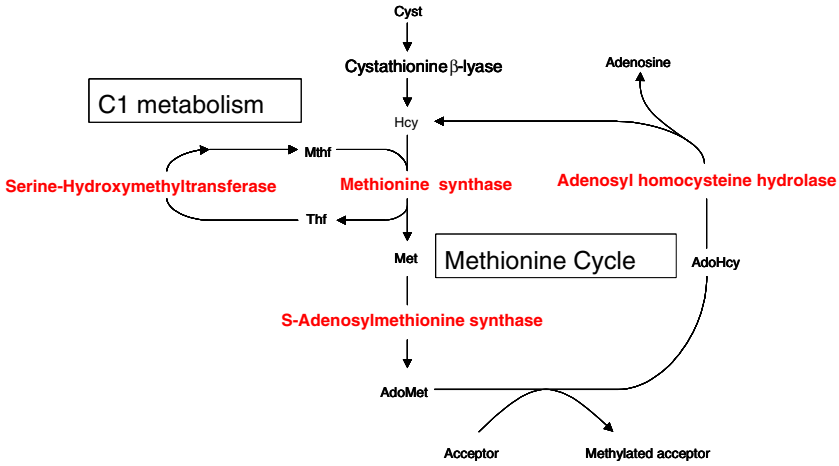

Fig. 2. Detection of integrated pathways in a plant specific cell type. Enzymes in red were found in trichome cells using nano LC/MS/MS analysis. The presence of the identified enzymes suggests a complete methionine cycle combined with C1 metabolism. Reaction intermediates: AdoHcy, S-adenosyl homocysteine; AdoMet, *S*-adenosylmethionine; Cyst, cystathionine; Hcy, homocysteine; Mthf, methyltetrahydrofolate; Thf, tetrahydrofolate.

conjugates are transported by a specific Mg^{2+} ATPase called glutathione S-conjugate translocator (Table 1) encoded by the *AtMRP1* gene, out of the cytosol into the vacuole (Lu et al., 1997). The presence of glutathione S-conjugate translocator supports the idea that trichomes might be involved in detoxification of xenobiotics and endogenous substances, including herbicides and anthocyanins (Gutierrez-Alcala et al., 2000).

3.4. Other proteins in trichome cells

We identified several additional proteins in trichome extracts (Table 1) such as acyl carrier protein 1 precursor (ACP), dnaK-type molecular chaperone hsc70, nucleoside-diphosphate kinase, Rubisco, enolase, ubiquitin-protein ligase (Vierstra, 2003), and others that are known to be ubiquitous in plants. Interestingly, phosphoenolpyruvate carboxykinase (PEPCK), which usually catalyses the reversible decarboxylation of oxaloacetate to yield phosphoenolpyruvate and CO_2 has been identified in this study (Table 1). Chen et al. (2000) reported that throughout senescence PEPCK is exclusively present in the trichomes and vasculature. Furthermore it has been suggested that PEPCK of trichomes is not involved in gluconeogenesis, but may be related to nitrogen metabolism because large amounts of NH_4^+ are released by the shikimate pathway, which is very active in many trichomes (Gang et al., 2002).

3.5. Unknown and hypothetical proteins found in trichome cells

A number of matches in the database led to hypothetical proteins with unknown or hypothetical functions. Due to the pure trichome extract analysed in this study, some identified proteins potentially represent novel trichome specific proteins. Hypothetical or unknown proteins were further analysed by sending them to a functional protein search (http://www.bork.embl-heidelberg.de/STRING/). This kind of orthogonal homology search provides further information on proteins in terms of putative domain structures or interaction partners known in other organisms. We systematically sent all the unknown/hypothetical proteins through this search. All domain information can be found at this address. Here, only one positive hit is found giving more information based on protein domain structure. At4g02660 is a putative transport protein with a BEACH domain usually followed by a series of WD repeats. The function of the BEACH domain is unknown but it appears to be crucial to the function of a large group of eukaryotic proteins involved in vesicle trafficking, membrane dynamics and receptor signalling (Jogl et al., 2002).

3.6. Differential analysis of epidermal cell proteins and trichome proteins

To determine if the method described here is applicable to other cell types, we also performed protein analysis in epidermal cells. Different protein profiles are a clear indication for a changed protein matrix and protein abundance (Greenbaum et al., 2003) and, thus, characteristic for specific cells. Epidermal cells were harvested following a protocol developed by Brandt et al. (1999). Glass capillaries with tip apertures of 1–10 μm were used to sample cell sap from epidermal cells. Sap of about 1000 cells was collected in trypsin buffer as described for trichome cells. Total protein amount could not be accurately measured but was estimated to be

Table 2
List of identified epidermis proteins

Accession no.	Protein [matched peptides (Mascot)]	Found with	Protein score (Mascot)	Xcorr (Sequest) [charge state]
At1g67090	Ribulose-bisphosphate carboxylase small unit [5]	Mascot/Sequest	159	3.23 [2+]
At4g21280	Oxygen-evolving complex protein 16, chloroplast precursor (OEC16) [2]	Mascot/Sequest	84	3.02 [2+]
At3g60860	Guanine nucleotide exchange factor-like	Mascot/Sequest	82	1.54 [2+]
At4g09000	14-3-3 protein GF14 chi (grf1) [3]	Mascot/Sequest	79	2.46 [2+]
At2g20260	Putative photosystem I reaction center subunit IV [2]	Mascot/Sequest	67	2.36 [2+]
At1g50250	Chloroplast FtsH protease [5]	Mascot/Sequest	53	2.05 [2+]
At1g13930	Unknown protein [1]	Mascot/Sequest	50	4.84 [3+]
At3g25510	Disease resistance protein (TIR-NBS-LRR class), putative [7]	Mascot	77	
At3g28770	Unknown protein	Mascot	74	
At3g19170	Metalloprotease, putative [6]	Mascot	74	
At5g04140	Ferredoxin-dependent glutamate synthase [10]	Mascot	72	
At4g27010	Unknown protein [10]	Mascot	70	
At2g07180	Putative protein kinase [5]	Mascot	69	
At5g52850	Selenium-binding protein-like [8]	Mascot	69	
At2g25550	Putative non-LTR retro-element reverse transcriptase [8]	Mascot	67	
At2g28290	Putative SNF2 subfamily transcription regulator [14]	Mascot	67	
At2g40930	Ubiquitin-specific protease 5 (UBP5), putative [7]	Mascot	66	
At5g65270	GTP-binding_protein,_putative	Sequest		2.196 [2+]
At3g57900	Unknown protein	Sequest		2.12 [2+]
At5g03410	DPB transcription factor	Sequest		2.06 [2+]
At1g13320	Protein_phosphatase_2A_regulatory_subunit,_putative	Sequest		1.93 [2+]
At3g42580	Unknown protein	Sequest		1.82 [2+]
At4g25800	Calmodulin-binding_protein	Sequest		1.65 [2+]
At4g10320	Isoleucine-tRNA_ligase_like_protein	Sequest		1.62 [2+]
At1g75040	Thaumatin-like protein	Sequest		1.57 [2+]
At5g15730	Serinethreonine-specific_protein_kinase-like_protein	Sequest		1.53 [2+]

much lower than 1 μg/1000 cells as determined for trichomes. Consequently, the average number of peptides identified for a specific protein as well as the total number of proteins is considerably lower. However, the initial analysis of epidermal cell proteins showed no overlap of identified proteins compared to trichome cell proteins (see Table 2). Thus, the specific cell protein profile seems to be significantly different. However, contamination with chloroplastidic proteins seems to be higher compared to the trichome protein profile (see Tables 1 and 2), which is in agreement with the more accurate sampling method for trichomes. Intensive studies comparing both cell proteomes will be necessary to fully characterise the role of these cell types. Nevertheless, there is no doubt about the applicability of this method to other cell types.

3.7. Conclusion

By continuing to apply single cell sampling and shotgun proteomics to specific plant cell types, a wealth of functional information will be garnered. The application of nano LC/MS/MS and novel sampling methods like laser capture micro dissection (Kehr, 2001; Nakazono et al., 2003) will increase sensitivity, and thus the number of identified proteins. However, higher amounts of proteins in smaller volumes and compatibility with instrumental set up are the critical issues to address.

For the first time it is possible to confirm previous suggestions about functions of trichomes via identified proteins supporting a role in sulfur metabolism and, more importantly, in detoxification (Lu et al., 1997; Gutierrez-Alcala et al., 2000). In addition to the worthwhile identification of specific cell protein components, further progress in method development, for instance differential stable isotope labelling (Gygi et al., 1999b; Oda et al., 1999; Weckwerth et al., 2000; Goodlett et al., 2001; Washburn et al., 2002; Gerber et al., 2003) or direct quantification in complex mixtures using LC/MS (Chelius and Bondarenko, 2002; Weckwerth et al., 2004), will enable relative or absolute protein quantification and cross-linking between physiological, genomic, metabolomic and proteomic data (Ideker et al., 2001; Griffin et al., 2002; Washburn et al., 2003; Weckwerth, 2003; Weckwerth et al., 2004).

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