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Transcriptional Profiling of Mature Arabidopsis Trichomes Reveals That NOECK Encodes the MIXTA-Like Transcriptional Regulator MYB106

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Leaf hairs (trichomes) of Arabidopsis (Arabidopsis thaliana) have been extensively used as a model to address general questions in cell and developmental biology. Here, we lay the foundation for a systems-level understanding of the biology of this model cell type by performing genome-wide gene expression analyses. We have identified 3,231 genes that are up-regulated in mature trichomes relative to leaves without trichomes, and we compared wild-type trichomes with two mutants, glabra3 and triphyllon, that affect trichome morphology and physiology in contrasting ways. We found that cell wall-related transcripts were particularly overrepresented in trichomes, consistent with their highly elaborated structure. In addition, trichome expression maps revealed high activities of anthocyanin, flavonoid, and glucosinolate pathways, indicative of the roles of trichomes in the biosynthesis of secondary compounds and defense. Interspecies comparisons revealed that Arabidopsis trichomes share many expressed genes with cotton (Gossypium hirsutum) fibers, making them an attractive model to study industrially important fibers. In addition to identifying physiological processes involved in the development of a specific cell type, we also demonstrated the utility of transcript profiling for identifying and analyzing regulatory gene function. One of the genes that are differentially expressed in fibers is the MYB transcription factor GhMYB25. A combination of transcript profiling and map-based cloning revealed that the NOECK gene of Arabidopsis encodes AtMYB106, a MIXTA-like transcription factor and homolog of cotton GhMYB25. However, in contrast to Antirrhinum, in which MIXTA promotes epidermal cell outgrowth, AtMYB106 appears to function as a repressor of cell outgrowth in Arabidopsis.

Organs of multicellular organisms comprise many different cell types at different developmental stages. For instance, just the epidermal tissue layer of mature Arabidopsis (Arabidopsis thaliana) leaves contains at least six morphologically distinct cell types: puzzle-shaped epidermal pavement cells in the central part of the leaf blade, long border cells at the margin, elongated epidermal cells overlying the midvein, guard cells comprising the stomata (pores), leaf hairs (trichomes), and trichome socket cells (Bowman, 1994). Each of these individual cell types is administered by a unique set of expressed genes, such as those that control the intricate structure of trichomes.

Trichomes can be found on most plants, and in some species they might play a role in protecting plants from insects by providing mechanical hindrance to the attackers. In addition, trichomes can reduce wind velocity and thus might reduce water loss through stomata by maintaining a highly water-saturated microenvironment over pores. In other species, trichomes might reflect excess light and thus protect the plant from radiation damage (Wagner et al., 2004).

Recent interest has arisen in trichomes as the location for the synthesis of secondary compounds. For instance,
stance, the aromatic substances in peppermint (\textit{Mentha piperita}) and basil (\textit{Ocimum basilicum}), including phenylpropanes and terpenoids, are synthesized in glandular trichomes (Lange et al., 2000; Iijima et al., 2004). In addition, trichomes are sites of detoxification (Gutiérrez-Alcalá et al., 2000) and are used by the plant as storage for heavy metals (Dominguez-Solis et al., 2004). The fibers of cotton (\textit{Gossypium hirsutum}), of major interest to the textile industry, are also trichomes. Fibers are formed on the seed coat of \textit{Gossypium} species and represent one of the most highly expanded cell types found in plants (Wilkins et al., 2000).

Due to their genetic accessibility, Arabidopsis trichomes have been used as a model cell type to study cell developmental processes (Folkers et al., 1997; Marks, 1997; Szymanski et al., 2000; Larkin et al., 2003; Martin and Glover, 2007; Schellmann et al., 2007). Trichome development in Arabidopsis represents an extreme case of anisotropic cell growth. The incipient trichome cell bulges out of the epidermal plane and then a second and third growth axis will be established, leading to a single cell with three limbs. To set up the secondary growth axes and their expansion, a tight organization of microtubules and microfilaments is required, and many mutants with altered trichome morphology have been isolated that are affected in the organization of their cytoskeleton (Hulskamp, 2000; Schellmann and Hulskamp, 2005; Szymanski, 2005).

During their outgrowth, Arabidopsis trichomes undergo approximately four rounds of endoreplication cycles, leading to final DNA content of 32C in the mature hair. As in many other cells, the amount of nuclear DNA has been found to correlate in general with trichome size; for instance, in \textit{glabra3} (\textit{gl3}) mutants, which display a reduction in nuclear DNA content to approximately 16C, the number of branches is reduced to two along with a general reduction of trichome size (Hulskamp et al., 1994; Schnittger et al., 2003). Conversely, in mutants like \textit{triphyction} (\textit{try}), which has an increased nuclear DNA content of approximately 64C, the number of branches is increased and the overall trichome size is larger than in the wild type (Hulskamp et al., 1994; Schnittger et al., 1999).

Complementing evidence that the level of endoreplication influences cell size and branch formation came from the ectopic expression of the cyclin-dependent kinase (CDK) inhibitor INHIBITOR/INTERACTOR OF CDK1/KIP-RELATED PROTEIN1 (ICK1/KRP1) in trichomes (Schnittger et al., 2003). The \textit{ICK1/KRP1}-misexpressing plants displayed a strongly reduced DNA content in trichomes, consistent with a requirement for CDK activity to drive the endoreplication cycle. Importantly, trichomes of these plants displayed fewer branches and were smaller than wild-type trichomes. A similar trichome phenotype was recently found in weak \textit{cdka1} mutants, in which the central regulatory CDK, CDKA1, is severely compromised (Dissmeyer et al., 2007).

In addition to this DNA-dependent influence on trichome growth and differentiation, there is also a DNA-independent component (Schnittger et al., 2003). This DNA-independent growth pathway is genetically defined by the \textit{noeck} (\textit{nok}) mutant, in which trichomes produce more branches but display no increase in nuclear DNA content (Folkers et al., 1997). However, very little is known about this DNA-independent effect on trichome cell shape.

Here, we present transcript profiles for mature wild-type Arabidopsis trichomes and for two mutants, \textit{gl3} and \textit{try}, with contrasting trichome phenotypes. The transcriptome gave a genome-wide insight into the development and metabolism of trichomes, in particular revealing high activity of the anthocyanin, flavonoid, and glucosinolate biosynthetic pathways, as well as the pathways for cell wall biosynthesis and lipid biosynthesis. As an example of the potential of our data set, we have used these data in combination with a map-based cloning approach to show that NOK, previously known only from mutants having trichomes with increased branching and a glassy transparent appearance, encodes the putative transcription factor MYB106 (At3g01140), which is closely related to MIXTA from \textit{Antirrhinum}. This highlights the importance of this subgroup of MYB transcription factors in regulating the outgrowth of plant epidermal cells and demonstrates the utility of the Arabidopsis trichome transcriptome.

**RESULTS**

**Expression Profile of Mature Wild-Type Trichomes**

Transcript profiling is a powerful tool for investigating cellular specification and function. However, most of the transcript profiles have been made from tissues containing several types of cells (e.g. whole seedlings or individual organs). Production of transcript profiles for single cell types can provide deeper insights but requires that the cells of interest can be separated from the rest of the tissue. So far, only a few single cell type expression profiling studies have been carried out in plants, such as protoplasting coupled to fluorescent cell sorting of root cells or direct harvesting of single-celled pollen grains (Becker et al., 2003; Birnbaum et al., 2003; Honys and Twell, 2003; Leonhardt et al., 2004; Aziz et al., 2005; Nayw et al., 2005; Brady et al., 2007).

Here, we took advantage of the large size of Arabidopsis trichomes and collected mature trichomes manually. Each wild-type trichome yielded an average of 0.1 ng of total RNA; therefore, a few hundred cells produced enough RNA for an Affymetrix GeneChip hybridization. To identify genes with an expression profile specific for trichomes, we compared the expression in trichomes (T sample) with a control set generated from leaves whose trichomes had been removed (LwoTsample) using three biological replicates of each.

To reduce technical noise, we focused on probe sets with a maximum 2-fold difference within the three trichome replicates. A total of 18,459 probe sets matched this criterion and were used for normalization and further analysis. The CEL files and the combined nor-
nalized data file are available at ArrayExpress with the accession number E-ATMX-33.

A critical question is the minimum expression level (cutoff value) used to score a gene as expressed. Since trichomes are easily accessible and gene activity in trichomes is often obvious, a trichome-specific role or expression pattern has been reported for many genes. This allowed us to follow a strategy previously employed for the analysis of the root transcriptome (Birnbaum et al., 2003). First, we performed a literature and database search, identifying 48 genes that have a trichome-specific mutant phenotype and/or are known to be expressed in trichomes (Supplemental Table S5). With the exception of GL3, all of these genes are represented on the Affymetrix ATH-1 chip.

A subset of these genes had identified roles or expression in mature trichomes, including GL2, CAPRICE (CPC), and the recently identified regulator of endoreplication and cell division, SIAMESE (SIM; Wada et al., 1997; Szymanski et al., 1998; Walker et al., 2000; Churchman et al., 2006). Conversely, others were known to be involved in early steps of trichome development and to show little or no expression in mature trichomes, such as GL1 and TRANSPARENT TESTA GLABRA2 (TTG2). Therefore, we set the cutoff value for trichome expression in such a way that the mature trichome genes were included but those specific to early trichome development were excluded. This resulted in a cutoff value of 50 arbitrary expression units (linear scale) and 5,461 leftover genes in the trichome data set.

Of these, 3,231 genes showed higher or equal expression levels in trichomes compared with leaves without trichomes (Supplemental Table S1). To create a trichome-specific gene set, genes that also showed expression in leaves without trichomes were removed; we set 30 arbitrary units as an expression threshold in the LwoT sample. This resulted in a core set of 1,115 trichome-specific genes (marked by asterisks in the Arabidopsis Genome Initiative code column in Supplemental Table S1). A list of the 5% most up-regulated genes in mature Arabidopsis trichomes is shown in Table I.

To characterize biological processes specific for trichomes, the Gene Ontology (GO) was determined for all genes up-regulated more than 2-fold (612 of 1,115 trichome-specific genes). The resulting networks of functional categories were visualized using BiNGO software (Fig. 1; Maere et al., 2005). Among genes with roles in cell physiology, those involved in stress responses, cell wall biosynthesis, and wax production were particularly overrepresented in trichomes; genes with developmental roles, in particular those known to play a role during atrichoblast patterning, were also found (see below).

Conversely, we analyzed the 250 most down-regulated genes for their GO (Supplemental Fig. S1). Genes from the categories of cuticle development and response to UV light (i.e. functions that are typical for epidermal cells) are overrepresented among the down-regulated genes. Furthermore, chloroplasts are not present in trichomes, and genes in the categories belonging to different aspects of photosynthesis (chlorophyll biosynthesis, photosynthetic electron transport, L-ascorbic acid biosynthesis, ammonia assimilation, plastid biogenesis, etc.) are among the most underrepresented genes. This shows that the preparation of trichomes for the array hybridization was not contaminated with mesophyll cells.

**Biosynthetic Pathways Up-Regulated in Trichomes**

Many specific functions have been ascribed to trichomes, including protection against herbivores and UV light, storage of toxic metal ions, and increased freezing tolerance. The GO annotation (Fig. 1) of transcripts in trichomes showed an overrepresentation of genes from the GO classes of responses to environmental and biotic stimuli. In addition, we used the AraCyc tool at The Arabidopsis Information Resource (release 4.0; www.arabidopsis.org/biocyc/index.jsp) to search for biosynthetic pathways involved in these functions that are specifically up-regulated. The protective function of trichomes was supported by their expression of the pathways for biosynthesis of a variety of glucosinolates and flavonoids, which act in plant defense and protection (Supplemental Table S6A; Supplemental Fig. S2).

In addition to the chemical defense, trichomes have been implicated in protecting the plant mechanically against herbivorous insects. Trichomes exhibit a very rigid cell wall, exemplified by the high mechanical and biochemical resistance against protoplasting (Zhang and Oppenheimer, 2004). This prompted us to analyze the expression profile for genes involved in cell wall biosynthesis. The AraCyc tool identified nine genes of the gluconegenes pathway that were up-regulated, producing hexose used in cell wall biosynthesis. In addition, genes belonging to the categories of cell wall and cellulose biosynthesis were detected. To get a more complete picture, we checked the approximately 900 candidate genes that might be involved in cell wall biosynthesis in Arabidopsis (Carpita et al., 2001; http://cellwall.genomics.purdue.edu/intro/index.html) for their expression in trichomes. Of these, 111 genes were found to be up-regulated in mature trichomes (Supplemental Table S6B), including the glucosyltransferase MURUS2 (MUR2), which is 2.64-fold up-regulated in trichomes. Remarkably, murr2 mutants have been reported to display defects in the development of papillae on the surface of trichomes (Vanzin et al., 2002).

In addition, we found the ARABINOGLACTAN PROTEIN4 (AGP4) to be 5.57-fold up-regulated in trichomes, consistent with an enhancer trap line reported to show GUS marker gene activity in trichomes (Cold Spring Harbor Laboratory gene trap line GT5714; Martienssen, 1998). However, two transposon-tagged mutant lines for AGP4 did not show a mutant trichome phenotype, presumably due to functional redundancy with paralogous genes (Supplemental Table S3).

Epidermal cells of plants are usually covered by a layer of cutin polymer and waxes, and several mutants that are compromised in the production of cuticular
Table I. The 5% most highly expressed genes in mature Arabidopsis trichomes

| pVAL | FC mT/mLwoT | Gene Title                                                                 | Arabidopsis Genome Initiative Code | FC mgl3-3/mT | FC mtry-JC/mT |
|------|-------------|-----------------------------------------------------------------------------|-----------------------------------|--------------|--------------|
| 0.07 | 109.20      | Expressed protein                                                           | AT3G18170                         | 1.05         | 1.68         |
| 0.03 | 99.37       | GLABRA2                                                                     | AT1G78940                         | 0.34         | 1.07         |
| 0.03 | 75.50       | Protein kinase family protein                                               | AT1G66460                         | 0.11         | 0.95         |
| 0.03 | 60.21       | Metal transporter, putative (ZIP7)                                          | AT2G04032                         | 1.07         | 2.09         |
| 0.01 | 58.81       | Expressed protein                                                           | AT3G19660                         | 0.45         | 0.52         |
| 0.08 | 48.31       | Glycosyl hydrolase family 17 protein                                        | AT3G04010                         | 1.30         | 1.43         |
| 0.01 | 47.47       | Low-temperature- and salt-responsive protein                               | AT4G30650                         | 0.14         | 0.34         |
| 0.01 | 45.95       | Expressed protein                                                           | AT1G22890                         | 0.62         | 1.51         |
| 0.00 | 45.16       | Pectinesterase family protein                                               | AT3G59010                         | 0.16         | 0.73         |
| 0.02 | 43.84       | 17.6-kD class I small heat shock protein (HSP17.6C-CI; amino acids 1–156)  | AT1G53540                         | 0.23         | 0.14         |
| 0.05 | 43.37       | Exocyst subunit EXO70 family protein                                        | AT3G05920                         | 0.63         | 1.94         |
| 0.03 | 41.03       | Hairpin-responsive protein, putative (HIN1)                                 | AT5G06330                         | 0.49         | 1.14         |
| 0.00 | 40.37       | Phosphoenolpyruvate carboxykinase, putative                                 | AT4G37870                         | 0.31         | 0.96         |
| 0.03 | 37.27       | Anion-exchange family protein                                               | AT1G74810                         | 0.61         | 1.44         |
| 0.00 | 33.79       | Gly-rich protein                                                            | AT4G29030                         | 0.52         | 0.38         |
| 0.00 | 31.59       | β-Galactosidase, putative/lactase, putative                                 | AT1G45130                         | 0.47         | 0.63         |
| 0.00 | 28.77       | Annexin 1 (ANN1)                                                            | AT1G35720                         | 0.51         | 0.69         |
| 0.02 | 27.96       | SIAMESE                                                                     | AT5G04470                         | 0.23         | 1.16         |
| 0.00 | 27.25       | Zinc finger (B-box type) family protein                                     | AT2G47890                         | 0.30         | 0.36         |
| 0.03 | 25.81       | Expressed protein                                                           | AT5G12420                         | 0.47         | 1.88         |
| 0.04 | 25.54       | Stricostidine synthase family protein                                       | AT1G74020                         | 0.50         | 1.89         |
| 0.01 | 24.11       | Fringe-related protein                                                      | AT5G41460                         | 0.56         | 0.85         |
| 0.04 | 23.67       | Phosphoinositide-specific phospholipase C (PLC1)                             | AT5G58670                         | 0.62         | 2.37         |
| 0.02 | 23.04       | Hairpin-induced family protein                                              | AT5G06320                         | 1.11         | 2.11         |
| 0.01 | 22.74       | Stricostidine synthase family protein                                       | AT1G74010                         | 0.83         | 0.69         |
| 0.00 | 22.50       | Hydrolase, α/β-fold family protein                                          | AT3G10870                         | 0.78         | 0.76         |
| 0.00 | 22.48       | Diacylglycerol O-acyltransferase/acyl-CoA: diacylglycerol acyltransferase    | AT2G19450                         | 0.45         | 0.50         |
| 0.01 | 22.23       | Flavonol synthase 1 (FLS1)                                                  | AT5G08640                         | 0.49         | 0.36         |
| 0.05 | 21.72       | Transferase family protein                                                  | AT1G65450                         | 0.38         | 0.93         |
| 0.00 | 21.47       | DRE-binding transcription factor, putative                                 | AT4G16750                         | 0.37         | 0.61         |
| 0.04 | 21.18       | Multicopper oxidase type I family protein                                   | AT3G13400                         | 1.04         | 1.97         |
| 0.13 | 21.13       | WRKY8Y                                                                      | AT5G64350                         | 0.96         | 1.95         |
| 0.05 | 21.05       | Expressed protein                                                           | AT1G33340                         | 1.20         | 1.39         |
| 0.00 | 20.59       | Zinc finger (C2H2-type) family protein                                      | AT2G28710                         | 0.69         | 0.42         |
| 0.01 | 20.13       | Fatty acid desaturase family protein                                        | AT1G06100                         | 0.84         | 1.34         |
| 0.05 | 19.98       | GDSL-motif lipase/hydrolase family protein                                  | AT5G33370                         | 0.47         | 1.08         |
| 0.12 | 19.53       | Inward-rectifying potassium channel, putative (KAT3, AKT4, KC1)            | AT4G32650                         | 0.51         | 1.73         |
| 0.00 | 19.13       | Integral membrane family protein                                            | AT2G36100                         | 0.35         | 0.27         |
| 0.01 | 19.02       | Polygalacturonase, putative/pectinase, putative                            | AT1G80170                         | 0.66         | 1.20         |
| 0.00 | 17.98       | Cytochrome b6, putative                                                     | AT2G46650                         | 0.75         | 0.26         |
| 0.00 | 17.14       | Jacalin lectin family protein                                               | AT3G16470                         | 0.88         | 0.34         |
| 0.00 | 16.90       | Lipid transfer protein 6 (LTP6)                                             | AT3G08770                         | 0.61         | 0.65         |
| 0.00 | 16.67       | α-Lactate dehydrogenase, putative                                           | AT4G17260                         | 0.30         | 0.60         |
| 0.10 | 16.48       | Peroxidase, putative/pectinase 33                                          | AT3G49120                         | 2.18         | 3.44         |
| 0.00 | 16.45       | Hydrolase, α/β-fold family protein                                          | AT2G39400                         | 0.57         | 0.61         |
| 0.02 | 15.93       | Expressed protein                                                           | AT5G61340                         | 0.28         | 0.73         |
| 0.14 | 15.88       | NAD-dependent epimerase/dehydratase family protein                          | AT2G24850                         | 0.49         | 1.51         |
| 0.04 | 14.43       | Integral membrane transporter family protein                                | AT1G64990                         | 0.84         | 1.31         |
| 0.04 | 13.98       | Expressed protein                                                           | AT4G22270                         | 0.51         | 1.66         |
| 0.04 | 13.80       | SEC14 cytosolic factor family protein/photophosphoglyceride transfer family | AT1G22180                         | 0.66         | 1.30         |
| 0.02 | 13.75       | Dormancy/auxin-associated family protein                                    | AT2G33830                         | 0.17         | 0.21         |
| 0.17 | 13.58       | Atmyb5                                                                      | AT3G13540                         | 1.32         | 1.77         |

(Table continues on following page.)
Table 1. (Continued from previous page.)

| pVAL | FC mT/mLwoT | Gene Title                                                                 | Arabidopsis Genome Initiative Code | FC mgL-3/mT | FC mtry-3/mT |
|------|-------------|-----------------------------------------------------------------------------|------------------------------------|-------------|-------------|
| 0.02 | 12.22       | Dehydrin xero2 (XERO2)/low-temperature-induced protein LI30 (LI310)          | AT3G50970                          | 0.48        | 1.13        |
| 0.04 | 12.21       | Fasciclin-like arabinoalactan protein (FLA7)                                 | AT2G04780                          | 0.97        | 2.29        |
| 0.10 | 11.98       | Proton-dependent oligopeptide transport (POT) family protein                 | AT3G01350                          | 0.37        | 1.15        |
| 0.06 | 11.93       | Expressed protein                                                            | AT5G42900                          | 0.05        | 0.11        |
| 0.08 | 11.93       | Hyp-rich glycoprotein family protein                                          | AT5G65660                          | 0.65        | 3.04        |
| 0.01 | 11.92       | 17.6-kD class I heat shock protein (HSP17.6A-Cl)/17.8-kD class I heat shock protein (HSP17.8-Cl) | AT1G59860                          | 0.22        | 0.10        |
| 0.07 | 11.86       | DNAJ heat shock N-terminal domain-containing protein                         | AT5G03030                          | 0.41        | 2.28        |
| 0.00 | 11.50       | Inorganic pyrophosphatase, putative (soluble)/pyrophosphate phosphohydrolase, putative/PPase, putative | AT3G53620                          | 0.25        | 0.30        |
| 0.02 | 11.43       | Cys proteinase RD19a (RD19A)/thiol protease                                 | AT4G39090                          | 0.96        | 1.46        |
| 0.07 | 11.28       | Expressed protein                                                            | AT3G14850                          | 0.75        | 1.01        |
| 0.00 | 11.13       | Speckle-type PO2 protein-related                                              | AT3G46360                          | 0.24        | 0.08        |
| 0.09 | 11.09       | Expressed protein                                                            | AT5G54530                          | 0.54        | 0.96        |
| 0.03 | 11.02       | Glycosyl transferase family 8 protein                                        | AT5G15470                          | 0.43        | 1.01        |
| 0.02 | 10.91       | Late embryogenesis abundant protein, putative/LEA protein, putative           | AT2G46140                          | 0.41        | 1.64        |
| 0.00 | 10.90       | Ser carboxypeptidase III, putative                                           | AT3G10410                          | 0.44        | 0.23        |
| 0.05 | 10.47       | Major intrinsic family protein                                               | AT4G17340                          | 0.74        | 2.46        |
| 0.01 | 9.98        | Pectinesterase family protein                                                | AT3G43270                          | 0.29        | 0.64        |
| 0.03 | 9.81        | Protein kinase family protein                                                | AT3G17420                          | 0.37        | 1.21        |
| 0.05 | 9.81        | Ser/Thr protein phosphatase, putative                                        | AT3G05580                          | 0.50        | 0.95        |
| 0.00 | 9.73        | Chalcone synthase/naringenin-chalcone synthase                               | AT5G13930                          | 0.66        | 0.25        |
| 0.00 | 9.71        | Pectinesterase family protein                                                | AT3G14310                          | 0.44        | 0.44        |
| 0.00 | 9.63        | Mitochondrial substrate carrier family protein                               | AT2G22500                          | 0.69        | 0.50        |
| 0.00 | 9.51        | Glycosyl hydrolase family 1 protein                                          | AT3G09260                          | 1.56        | 0.62        |
| 0.03 | 9.50        | Expressed protein                                                            | AT3G61840                          | 0.10        | 0.73        |
| 0.03 | 9.42        | Expressed protein                                                            | AT1G31200                          | 0.54        | 1.10        |
| 0.11 | 9.28        | CAPRICE                                                                     | AT2G46140                          | 0.71        | 2.40        |
| 0.03 | 9.17        | Expressed protein                                                            | AT1G52910                          | 0.78        | 1.02        |
| 0.06 | 9.15        | Potassium channel tetramerization domain-containing protein                  | AT2G24240                          | 0.68        | 0.72        |
| 0.00 | 9.10        | Ethylene-responsive element-binding factor 2 (ERF2)                          | AT5G47220                          | 0.50        | 0.33        |
| 0.00 | 9.01        | Galactinol synthase, putative                                                | AT3G28340                          | 0.67        | 0.58        |
| 0.12 | 8.93        | Auxin-responsive protein-related                                             | AT5G20820                          | 0.63        | 2.13        |
| 0.02 | 8.74        | Jacalin lectin family protein                                                | AT3G16450                          | 1.58        | 0.74        |
| 0.07 | 8.74        | Exostosin family protein                                                     | AT2G28110                          | 1.01        | 0.91        |
| 0.00 | 8.70        | Ca2+-dependent nuclease                                                      | AT3G56170                          | 0.83        | 0.61        |
| 0.01 | 8.65        | Sugar transporter, putative                                                  | AT1G08920                          | 0.42        | 1.32        |
| 0.00 | 8.62        | Matrixin family protein                                                      | AT1G59957                          | 0.46        | 0.96        |
| 0.10 | 8.44        | Cytochrome P450, putative                                                   | AT3G10570                          | 0.48        | 0.91        |
| 0.00 | 8.43        | Expressed protein/expressed protein                                          | AT5G10695                          | 0.99        | 0.73        |
| 0.00 | 8.38        | Histone H3                                                                  | AT1G09200                          | 0.73        | 0.34        |
| 0.01 | 8.28        | Major latex protein-related/MLP-related                                       | AT1G70890                          | 1.62        | 0.69        |
| 0.05 | 7.99        | Jacalin lectin family protein                                                | AT2G39310                          | 1.87        | 0.64        |
| 0.00 | 7.72        | Hydrophobic protein, putative/low-temperature- and salt-responsive protein, putative | AT4G30660                          | 0.35        | 0.27        |
| 0.01 | 7.68        | Expressed protein                                                            | AT2G15890                          | 0.34        | 0.15        |
| 0.01 | 7.64        | Hyp-rich glycoprotein family protein                                         | AT1G23040                          | 0.81        | 1.29        |
| 0.00 | 7.53        | Expressed protein                                                            | AT5G06270                          | 0.35        | 0.45        |
| 0.00 | 7.51        | Ethylene-responsive element-binding protein 1 (ERF1)/EREBP-2 protein         | AT4G17500                          | 0.47        | 0.32        |
| 0.00 | 7.49        | Bet v 1 allergen family protein                                              | AT1G24020                          | 1.20        | 0.86        |
| 0.00 | 7.47        | Superoxide dismutase (Cu-Zn; SODCC)/copper-zinc superoxide dismutase (CSD1) | AT1G08830                          | 1.25        | 1.18        |
| 0.02 | 7.40        | SEC14 cytosolic factor (SEC14)/phosphoglyceride transfer protein             | AT1G55840                          | 0.67        | 1.57        |

(Table continues on following page.)
Table 1. (Continued from previous page.)

| pVAL | FC mT/mLwoT | Gene Title | Arabidopsis Genome Initiative Code | FC mgB-3/mT | FC mty-J/mT |
|------|-------------|------------|-----------------------------------|-------------|-------------|
| 0.08 | 7.37        | Expressed protein | AT5G19250 | 0.98 | 2.69 |
| 0.05 | 7.32        | Expressed protein | AT3G21700 | 0.39 | 2.02 |
| 0.01 | 7.25        | Cytochrome P450, putative | AT1G01600 | 0.66 | 1.10 |
| 0.01 | 7.10        | Expressed protein | AT3G50350 | 0.23 | 1.37 |
| 0.07 | 6.93        | Late embryogenesis abundant protein, putative/LEA protein, putative | AT1G01470 | 0.70 | 2.14 |
| 0.00 | 6.92        | Protein kinase family protein | AT2G25220 | 0.84 | 0.99 |
| 0.01 | 6.82        | Aldose 1-epimerase family protein | AT3G17940 | 0.73 | 1.30 |
| 0.02 | 6.76        | Calcium-binding mitochondrial protein-related | AT3G59820 | 0.56 | 0.63 |
| 0.00 | 6.75        | Mitochondrial import inner membrane translocase subunit Tim17/Tim22/Tim23 family protein | AT4G26670 | 0.35 | 0.29 |
| 0.01 | 6.73        | Homeodomain transcription factor (KNAT7) | AT1G62990 | 0.83 | 1.09 |
| 0.02 | 6.69        | Expressed protein | AT5G63500 | 0.61 | 1.16 |
| 0.00 | 6.69        | Malate dehydrogenase (NAD), mitochondrial, putative | AT3G15020 | 0.61 | 0.20 |
| 0.00 | 6.60        | Fasciclin-like arabinogalactan protein, putative | ATSG44130 | 0.37 | 0.76 |
| 0.00 | 6.58        | Expressed protein | AT1G27290 | 0.36 | 0.68 |
| 0.03 | 6.57        | Ras-related GTP-binding protein, putative | AT4G17160 | 0.47 | 1.15 |
| 0.00 | 6.56        | Expressed protein | AT3G10020 | 0.33 | 0.17 |
| 0.00 | 6.48        | Histone H2A, putative | AT5G59870 | 0.87 | 0.71 |
| 0.00 | 6.47        | Pectinesterase family protein | AT5G45280 | 0.60 | 0.89 |
| 0.01 | 6.45        | Expressed protein | AT3G21190 | 0.32 | 1.50 |
| 0.04 | 6.45        | Drought-responsive protein/drought-induced protein (Di21) | AT4G15910 | 1.34 | 0.81 |
| 0.05 | 6.43        | Transferase family protein | AT5G39090 | 0.70 | 0.93 |
| 0.04 | 6.40        | Cys proteinase, putative/thiol protease, putative | AT5G43060 | 0.47 | 1.54 |
| 0.00 | 6.34        | Heavy metal-associated domain-containing protein/copper chaperone (CCH)-related | AT4G38580 | 0.35 | 0.24 |
| 0.00 | 6.32        | Alcohol dehydrogenase (ADH) | AT1G77120 | 0.45 | 0.43 |
| 0.01 | 6.23        | U-box domain-containing protein | AT2G35930 | 0.49 | 1.17 |
| 0.00 | 6.18        | Glutamate dehydrogenase 2 (GDH2) | AT5G07440 | 0.45 | 0.20 |
| 0.00 | 6.17        | Kelch repeat-containing F-box family protein | AT1G15670 | 0.48 | 0.45 |
| 0.00 | 6.07        | AP2 domain-containing transcription factor, putative | AT4G34410 | 0.44 | 0.19 |
| 0.00 | 6.07        | Dormancy/auxin-associated family protein | AT1G56220 | 0.23 | 0.21 |
| 0.04 | 5.96        | Hydrophobic protein (RC12A/low-temperature- and salt-responsive protein (LT6a) | AT3G05880 | 0.70 | 1.97 |
| 0.00 | 5.90        | SEC14 cytosolic factor family protein/phosphoglyceride transfer family protein | AT1G72160 | 0.62 | 1.00 |
| 0.00 | 5.84        | Expressed protein | AT5G18850 | 0.48 | 0.85 |
| 0.01 | 5.78        | AB3-interacting protein 1 (AIP1) | AT5G61380 | 0.27 | 0.36 |
| 0.01 | 5.75        | Expressed protein | AT1G76240 | 0.97 | 0.96 |
| 0.00 | 5.74        | Cell death-associated protein-related | AT1G49650 | 0.45 | 0.36 |
| 0.04 | 5.73        | Tubulin β-1 chain (TUB1) | AT1G75780 | 0.44 | 0.74 |
| 0.00 | 5.72        | Alcohol dehydrogenase, putative | AT1G64710 | 0.45 | 0.53 |
| 0.01 | 5.71        | No apical meristem (NAM) family protein | AT1G52880 | 1.03 | 1.17 |
| 0.11 | 5.70        | OTU-like Cys protease family protein | AT3G22260 | 1.19 | 1.73 |
| 0.00 | 5.62        | Adenine phosphoribosyltransferase 1 (APT1) | AT1G7450 | 0.87 | 0.88 |
| 0.01 | 5.59        | Transaldolase, putative | AT5G13420 | 0.69 | 0.55 |
| 0.00 | 5.57        | Nonspecific lipid transfer protein 1 (LTP1) | AT2G38540 | 0.58 | 0.36 |
| 0.00 | 5.57        | Cytochrome P450 family protein | AT4G33770 | 1.40 | 0.52 |
| 0.11 | 5.57        | Arabinogalactan protein (AGP4) | AT5G10430 | 0.62 | 3.60 |
| 0.00 | 5.51        | Expressed protein | AT3G57450 | 0.88 | 0.61 |
| 0.05 | 5.50        | Integral membrane family protein | AT4G15620 | 0.50 | 0.84 |
| 0.00 | 5.45        | Cys protease inhibitor, putative/cystatin, putative | AT3G12490 | 0.56 | 0.76 |
| 0.21 | 5.45        | Gic transporter, putative | AT4G21480 | 0.54 | 1.21 |
| 0.02 | 5.44        | Trehalose-6-P phosphatase, putative | AT4G12430 | 1.04 | 0.95 |
| 0.00 | 5.38        | Expressed protein | AT1G21010 | 0.59 | 0.62 |
| 0.01 | 5.38        | Lipoygenase (LOX1) | AT1G55020 | 0.87 | 0.70 |
| 0.03 | 5.37        | Myb family transcription factor NOK (MYB106) | AT3G01140 | 0.64 | 0.70 |
| 0.01 | 5.36        | Pectinesterase family protein | AT5G53370 | 0.62 | 1.26 |
| 0.00 | 5.35        | Lipid-associated family protein | AT4G39730 | 0.90 | 1.11 |

(Table continues on following page.)
waxes have been reported to have aberrant trichome phenotypes, such as ECERIFERUM2 (CER2; Xia et al., 1997), ECERIFERUM10 (Zheng et al., 2005), FIDDLEHEAD (Yephremov et al., 1999), LACERATA (Wellesen et al., 2001), and YORE-YORE (Kurata et al., 2003). However, of these, only CER2 showed a slight up-regulation in trichomes (1.13 higher in T versus LwoT).

To explore a possible function of other cuticular wax synthesis genes during trichome development, we analyzed the expression of the approximately 700 genes that are suspected to be involved in acyl lipid metabolism (Beisson et al., 2003). In trichomes, 41 of these genes were expressed at a more than 2-fold higher level than in leaves without trichomes (Supplemental Table S6C). Thus, enzymes involved in lipid metabolism are not particularly overrepresented in trichomes, but possibly due to the elaborated structure of trichomes and a high demand to stabilize the cell shape, slight alterations in cutin production already are reflected in altered trichome morphology.

Comparison between Trichomes and Atrichoblasts

In the trichome transcriptome, we found a strong enrichment for genes involved in root atrichoblast differentiation (Fig. 2A). This assignment matched our initial expectation, since trichome development in leaves and atrichoblast development in roots are known to share a network of transcription factors involved in pattern formation and early cell differentiation (Larkin et al., 2003; Pesch and Hulskamp, 2004; Schellmann et al., 2007). However, little is known about the downstream targets of these factors, and it is not clear whether trichomes and atrichoblasts share common patterns of gene expression.

To address at a genome-wide level which downstream factors are common to trichomes and atrichoblasts, we first identified atrichoblast-specific transcripts by calculating the reported expression in sorted PROGL2:GFP-expressing cells (atrichoblasts) over the mean for stages I + II + III of root development (for definitions, see Birnbaum et al., 2003). Of 2,772 genes that showed a signal higher than 30 and expression more than 1.2-fold higher in atrichoblast than in the root tip, 820 were also found in the trichome gene set (Supplemental Tables S2A and S7).

These genes were first analyzed for their GO annotation using the BiNGO software and for their participation in metabolic pathways using the AraCyc tool. Consistent with the above findings, trichomes and atrichoblasts share the functional GO category “regulation of trichoblast fate” represented by GL2 and CPC (Fig. 2A). In addition, an overlap in gene functions for both cell types was found in the categories of nucleosome assembly (histones) and response to external stimuli. However, secondary metabolite synthesis (flavonoid/anthocyanin biosynthesis) and the production of glucosinolates seem to be specific for trichomes.

Next, we asked whether the overlap in regulatory circuits involved in trichome and atrichoblast development is reflected in a global similarity of expressed genes. The similarity of the expression signature of wild-type trichomes to atrichoblasts was compared with the similarity between trichomes and other root cell types of stele, endodermis, and cortex and root cap cells (Birnbaum et al., 2003). Remarkably, 820 genes were found to be similarly up-regulated in both atrichoblasts and trichomes, while each of the root cell types shared only approximately half this number of up-regulated genes with trichomes (Fig. 2B; Supplemental Table S2). This indicates that many of the genes that are known to pattern both trichomes and atrichoblasts also control later differentiation processes, resulting in a global similarity between these two cell types.

Transcriptome of gl3 and try Mutants

Mature wild-type trichomes are usually three branched and have a DNA content of about 32C. Several mutants have been identified in which the cell morphology and DNA content are altered. To gain insight into the underlying molecular mechanisms of these morphological changes, we carried out transcript profiling of the underbranched and underreplicating gl3 mutant and the overbranched and overreplicating try mutant. try mutants also display a patterning defect with clustering of trichomes (Hulskamp et al., 1994). Both TRY and GL3 encode transcription factors that regulate trichome cell fate (Payne et al., 2000; Zhang et al., 2003; Schellmann et al., 2007).

In our analysis, we focused on the differentially expressed genes previously defined by comparing wild-
type trichomes (T) with leaves without trichomes (LwoT; Supplemental Table S1A). Three known regulators of trichome development showed clear differences in their expression levels in gl3 and/or try mutant trichomes (Table II). A putative direct target of GL3 is the transcription factor GL2 (Morohashi et al., 2007; Zhao et al., 2008), and we also observed here that GL2 is down-regulated in gl3 trichomes. Conversely, the activity of a GL2 promoter reporter was found to be reduced in the root epidermis of gl3 mutants (Bernhardt et al., 2003). GL2 belongs to a group of 16 related homeodomain transcription factors, called HOMEO-DOMAIN GLABROUS (HDG; Nakamura et al., 2006). Among the HDGs, AT3G61150 (HDG1) shares the highest similarities with GL2 and is also expressed in trichomes. Since AT3G61150 was found here to be down-regulated in gl3 (0.48-fold), it could represent a target of GL3 action. However, HDG1 was also down-

Figure 1. GO analysis of genes that are 2-fold more highly expressed in trichomes than in leaves without trichomes. The size of a node within the network is proportional to the number of genes in the respective GO category. The level of significance of an overrepresented GO category is indicated by the shift from yellow to orange, corresponding to a P value from 5.00E-2 to <5.00E-7. Statistical testing was as described by Maere et al. (2005). [See online article for color version of this figure.]

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Figure 2. Comparison of expression profiles between trichomes and atrichoblasts. A, GO analysis of genes expressed in both trichomes and atrichoblasts. For annotation, see Figure 1. B, Venn diagram showing the overlap of expression of genes in trichomes and different cell types of the root. Please note that the overlap between the different root cell types cannot be presented here. [See online article for color version of this figure.]
Table II. The 25 most up- and down-regulated genes in gl3-3 and try-JC trichomes

| Gene Title                                                                 | Arabidopsis Genome Initiative Code | FC \(mT/mT\) |
|---------------------------------------------------------------------------|------------------------------------|--------------|
| The 25 most up-regulated genes in gl3-3 trichomes                         |                                     |              |
| Long-chain fatty acid CoA ligase, putative (LACS3)                         | AT1G64400                          | 2.86         |
| Pathogenesis-related protein 5 (PR-5)                                     | AT1G75040                          | 2.77         |
| Major latex protein-related                                               | AT4G23670                          | 2.72         |
| Peroxidase, putative/peroxidase 33 (PER33; P33; PRXCA)/neutral peroxidase C (PERC) | AT3G49120 /// AT3G49110            | 2.18         |
| Protein kinase, putative                                                  | AT1G76360                          | 2.05         |
| Outer envelope membrane protein, putative                                 | AT3G52420                          | 2.00         |
| Xyloglucan:xyloglucosyl transferase, putative/xyloglucan endotransglycosylase, putative/endoxyloglucan transferase, putative | AT4G03210                          | 1.99         |
| Wall-associated kinase 2 (WAK2)                                           | AT1G21270                          | 1.98         |
| Lectin protein kinase family protein                                       | AT4G02410                          | 1.88         |
| Jacalin lectin family protein                                             | AT2G39310                          | 1.87         |
| Expressed protein                                                         | AT2G25510                          | 1.85         |
| Short-chain dehydrogenase/reductase (SDR) family protein                  | AT3G03980                          | 1.84         |
| Wound-responsive protein-related/NADH-dehydrogenase-related               | AT4G28240 /// AT4G28220            | 1.83         |
| Expressed protein                                                         | AT3G49490                          | 1.82         |
| Zinc finger (C3HC4-type RING finger) family protein                       | AT4G30400                          | 1.82         |
| Callose synthase 1 (CALS1)/1,3-β-glucan synthase 1                        | AT1G05570                          | 1.78         |
| Jacalin lectin family protein                                             | AT3G16460                          | 1.78         |
| T-complex protein 11                                                       | AT1G22930                          | 1.77         |
| Ammonium transporter 2 (AMT2)                                              | AT2G38290                          | 1.77         |
| Expressed protein                                                         | AT2G27260                          | 1.76         |
| Lys- and His-specific transporter, putative                               | AT5G40780                          | 1.76         |
| Expressed protein                                                         | AT5G08320                          | 1.75         |
| Expressed protein                                                         | AT1G03290                          | 1.74         |
| Receptor-like protein kinase 4, putative (RLK4)                            | AT4G23180                          | 1.73         |
| The 25 most down-regulated genes in gl3-3 trichomes                       |                                     |              |
| Zinc finger (B-box-type) family protein                                    | AT2G47890                          | 0.30         |
| L-Lactate dehydrogenase, putative                                         | AT4G17260                          | 0.30         |
| Expressed protein                                                         | AT4G09950                          | 0.29         |
| Pectinesterase family protein                                             | AT3G43270                          | 0.29         |
| Hydrolase, α/β-fold family protein                                       | AT5G13800                          | 0.28         |
| Expressed protein                                                         | AT5G61340                          | 0.28         |
| Expressed protein                                                         | AT1G78890                          | 0.27         |
| ABI3-interacting protein 1 (API1)                                         | AT5G61380                          | 0.27         |
| Cold-responsive protein/cold-regulated protein (cor15b)                   | AT2G42530                          | 0.26         |
| Inorganic pyrophosphatase, putative (soluble)/pyrophosphate phosphohydrolase, putative/PPase, putative | AT3G53620                          | 0.25         |
| Expressed protein                                                         | AT1G17490                          | 0.24         |
| Speckle-type POZ protein-related                                          | AT3G48360                          | 0.24         |
| SIAMESE                                                                   | AT5G04470                          | 0.23         |
| Dormancy/auxin-associated family protein                                  | AT1G56220                          | 0.23         |
| Expressed protein                                                         | AT3G50350                          | 0.23         |
| 17.6-kD class 1 small heat shock protein (HSP17.6C-CI; amino acids 1–156) | AT1G53540                          | 0.23         |
| Senescence-associated protein (SEN1)                                       | AT4G35770                          | 0.23         |
| 17.6-kD class 1 heat shock protein (HSP17.6A-CI)                          | AT1G59860 /// AT1G207400           | 0.22         |
| Hypothetical protein                                                      | AT4G17860                          | 0.19         |
| Dormancy/auxin-associated family protein                                  | AT2G33830                          | 0.17         |
| Pectinesterase family protein                                             | AT3G59010                          | 0.16         |
| Hydrophobic protein, putative/low-temperature- and salt-responsive protein, putative | AT4G30650                          | 0.14         |
| Protein kinase family protein                                             | AT1G66460                          | 0.11         |
| Expressed protein                                                         | AT3G61840                          | 0.10         |
| Expressed protein                                                         | AT5G42900                          | 0.05         |

(Table continues on following page.)
Table II. (Continued from previous page.)

| Gene Title                                                                 | Arabidopsis Genome Initiative Code | FC mg/l-3-5/ mT |
|--------------------------------------------------------------------------|-----------------------------------|----------------|
| **The 25 most up-regulated genes in try-JC trichomes**                   |                                   |                |
| Arabinogalactan protein (AGP4)                                           | AT5G10430                         | 3.60           |
| Peroxinsomal protein PEX19 family protein                                | AT3G03490                         | 3.46           |
| Peroxidase, peroxidase 33 (PER33; PER33; PRXCA neutral peroxidase C (PERC) | AT3G49120 /// AT3G49110           | 3.44           |
| Expressed protein                                                        | AT2G03350                         | 3.35           |
| Hyp-rich glycoprotein family protein                                     | AT5G65660                         | 3.04           |
| Outer envelope membrane protein, putative                                | AT3G25420                         | 3.04           |
| Ndr family protein                                                       | AT5G11790                         | 3.00           |
| Hypothetical protein                                                     | AT4G16240                         | 2.94           |
| Zinc finger (C3HC4-type RING finger) family protein                      | AT5G66070                         | 2.88           |
| Wound-responsive protein-related                                         | AT3G07230                         | 2.76           |
| Expressed protein                                                        | AT5G19250                         | 2.69           |
| Cytochrome P450, putative                                                | AT4G37310                         | 2.64           |
| FtsH protease, putative                                                  | AT1G07510                         | 2.54           |
| 3-β-Hydroxysteroid dehydrogenase/isomerase family protein               | AT2G43420                         | 2.49           |
| Expressed protein                                                        | AT5G13190                         | 2.48           |
| Casein kinase, putative                                                  | AT1G04440                         | 2.48           |
| Major intrinsic family protein/MIP family protein                        | AT4G17340                         | 2.46           |
| Zinc finger (AN1-like) family protein                                    | AT4G12040                         | 2.43           |
| Expressed protein                                                        | AT5G15320                         | 2.42           |
| Expressed protein                                                        | AT1G10410                         | 2.41           |
| Myb-related protein CAPRICE (CPC)                                        | AT2G46410                         | 2.40           |
| Expressed protein                                                        | AT1G02160                         | 2.37           |
| Phosphoinositide-specific phospholipase C (PLCI)                         | AT5G58670                         | 2.37           |
| Expressed protein                                                        | AT5G64130                         | 2.36           |
| Expressed protein                                                        | AT1G65720                         | 2.35           |
| **The 25 most down-regulated genes in try-JC trichomes**                 |                                   |                |
| Ethylene-responsive element-binding factor 4 (ERF4)                      | AT3G15210                         | 0.16           |
| Mitochondrial processing peptidase α-subunit, putative                   | AT1G39180                         | 0.16           |
| Geranylgeranyl pyrophosphate synthase, putative                          | AT4G34860                         | 0.15           |
| Senescence-associated protein (SEN1)                                     | AT4G35770                         | 0.15           |
| Elongation factor 1B α-subunit 1 (eEF1 Ba1)                              | AT5G12110                         | 0.15           |
| Expressed protein                                                        | AT5G15630                         | 0.15           |
| Expressed protein                                                        | AT2G15890                         | 0.15           |
| 17.6-kD class I small heat shock protein (HSP17.6C-CI; amino acids 1–156)| AT1G35540                         | 0.14           |
| Pyruvate kinase, putative                                                | AT5G63680                         | 0.14           |
| Alkyl hydroperoxide reductase/thiol-specific antioxidant (AbpC/TSA)     | AT3G06050                         | 0.13           |
| Hevein-like protein                                                      | AT3G04720                         | 0.13           |
| Gly cleavage system H protein, mitochondrial, putative                   | AT2G35120                         | 0.13           |
| 26S proteasome non-ATPase regulatory subunit 7, putative/26S proteasome regulatory subunit S12, putative/MOV34 protein, putative | AT5G05780 | 0.13 |
| Glutathione S-transferase (GST10)                                       | AT5G41210                         | 0.13           |
| Glycoside hydrolase starch-binding domain-containing protein             | AT5G26570                         | 0.12           |
| Transmembrane CLPTM1 family protein                                      | AT3G08500                         | 0.12           |
| Man-6-P reductase (NADPH-dependent), putative/Man-6-P reductase (NADPH-dependent), putative | AT2G21250 AT2G21260 /// AT2G21250 | 0.12 |
| Acyl-CoA oxidase (ACX2)                                                  | AT5G65110                         | 0.12           |
| Expressed protein                                                        | AT5G42900                         | 0.11           |
| 17.6-kD class I heat shock protein (HSP17.6A-CI)/17.8-kD class I heat shock protein (HSP17.8-CI) | AT1G39860 AT1G07400 | 0.10 |
| β-Ketoacyl-CoA synthase family protein                                   | AT1G07270                         | 0.09           |
| Transport protein particle (TRAPP) component Bet3 family protein         | AT3G50000                         | 0.09           |
| Root cap 1 (RCP1)                                                       | AT5G17520                         | 0.08           |
| Speckle-type POZ protein-related                                         | AT3G48360                         | 0.08           |
| RWD domain-containing protein                                            | AT1G51730                         | 0.05           |
| AP2 domain-containing protein RAP2.12 (RAP2.12)                          | AT1G33910                         | 0.04           |
regulated in try (0.73-fold) mutant trichomes, suggesting additional regulatory input different from that on GL2.

Similarly, we found that CPC is down-regulated in gl3 (0.71-fold) mutant trichomes. An equal dependence of CPC expression on GL3 has been identified in the root epidermis (Bernhardt et al., 2003). This is consistent with a model in which cell fate activators for trichomes and atrichoblasts like GL3 induce not only their own expression but also the expression of their repressors, such as CPC (Larkin et al., 2003). Indeed, we also found that the expression of CPC is up-regulated in try mutants (2.4-fold), consistent with more GL3 being synthesized and leading to more CPC transcript. This presumptive regulatory loop is supported by mutant analysis, and the cpe try double mutants display a dramatic increase of clustered trichomes (Schellmann et al., 2002). CPC and TRY belong to a small gene family of single repeat myb domain transcription factors, which also includes four other transcriptional regulators, designated ENHANCER OF TRY AND CPC1 (ETC1), ETC2, ETC3 (also called CPC-LIKE3 [CPL3]), and TRICHOMELESS1 (TCL1), which are involved in trichome patterning (Király et al., 2004a, 2004b; Simon et al., 2007; Wang et al., 2007; Tominaga et al., 2008). However, while ETC3 and TCL1 are not present on the Affymetrix array, ETC1 and ETC2 were neither expressed in mature wild-type trichomes nor differentially expressed in try or gl3 mutant trichomes, suggesting that they predominantly function during early trichome patterning.

Another likely target gene of GL3 is SIM, a putative CDK inhibitor involved in the control of endoreplication and cell division (Walker et al., 2000; Churchman et al., 2006). SIM was more weakly expressed in the under-replicated gl3 trichomes (0.23-fold) and slightly more strongly expressed in the overreplicated try mutant trichomes (1.16-fold) in comparison with mature wild-type trichomes. This expression behavior is consistent with transcript levels of SIM in response to altered GL3 expression levels previously obtained by quantitative reverse transcription-PCR (Churchman et al., 2006). In addition to SIM, seven SIM-related/EL2-like putative CDK inhibitors likely are present in Arabidopsis (Churchman et al., 2006; Peres et al., 2007). One of these genes, At3g10525 (SMRI), was found to be 1.23-fold up-regulated in mature trichomes in comparison with leaves without trichomes, and similar to SIM, its expression was enhanced in a try mutant background (1.68-fold), suggesting an overlapping function with SIM during trichome development.

Taken together, our expression analysis reproduces some of the few known regulatory circuits. Thus, other genes differentially expressed in gl3 and/or try are candidates for targets of the respective transcriptional regulators, especially if transcripts are reciprocally regulated, as is the case for a putative peptide encoded by At1g22890 that is down-regulated in gl3 and up-regulated in try trichomes (Table II; Supplemental Table S1A).

Comparison of Arabidopsis Trichomes with Cotton Fibers

Cotton fibers are trichomes growing on the outer integument of cotton ovules. It is tempting to speculate that cotton fibers and Arabidopsis trichomes may share developmental programs in order to create these cells protruding from the epidermis, especially since the Malvaceae and the Brassicaceae are closely related plant families.

We looked for the overlap of our core trichome gene set with genes that are highly expressed in cotton fibers (Arpat et al., 2004). Of the 45 most highly expressed genes in a cotton fiber EST library, after BLAST search 44 had identifiable Arabidopsis homologs and 36 of these were represented in the Arabidopsis trichome data set (Supplemental Table S8A). Of 66 genes that were significantly up-regulated in expanding compared with emerging cotton fibers and that also had identifiable Arabidopsis homologs, 53 were also over-represented in Arabidopsis trichomes relative to leaves without trichomes (Supplemental Table S8B). These findings demonstrate the high overlap in the gene functions necessary for Arabidopsis trichome and cotton fiber development.

In a comparison of the expression profiles of the outer integuments of wild-type cotton ovules with those of lintless cotton mutants, 11 genes were identified to be differentially expressed (Wu et al., 2006). No homologs were detected for two of these genes in Arabidopsis. Of the remainder, via BLAST search, homologues of six cotton genes were identified and also found to be differentially expressed in Arabidopsis (Supplemental Table S8C). One of those genes was the Myb transcription factor GlMyb25, whose ortholog AtMYB106 was found to be of major importance for Arabidopsis trichome development (see below). The high overlap in the gene functions used for trichome development in both cotton and Arabidopsis highlights the value of this model system to investigate certain traits of economically important crop plants.

Functional Analysis of At1g66460 and WRKY8 (At5g46350)

Transcriptional profiling has the potential to pinpoint previously unrecognized regulators of biological processes. This has successfully been applied to stoma patterning (Leonhardt et al., 2004) and root hair development (Jones et al., 2006). Therefore, we analyzed in more detail some of the genes that showed a highly up-regulated expression in trichomes.

Two genes were initially selected for further analysis, a putative protein kinase (At1g66460), which is 75.5-fold more highly expressed in trichomes than in leaves, and WRKY8 (At5g46350), which was chosen because of its high differential expression (21-fold stronger expression in trichomes than in leaves without trichomes) and the known involvement of TTG2, a WRKY-type transcription factor in trichome development (Johnson et al., 2002).
Trichome-specific promoter activity was confirmed for both genes using approximately 2 kb of upstream sequences to control the expression of a GUS reporter gene (Fig. 3). For At1g66460, expression in leaves was confined to trichomes, while in the roots no expression was detected in atrichoblasts, although a weak and somewhat patchy expression was seen in lateral root cap cells (Fig. 3C). For WRKY8, strong GUS activity was observed in the trichomes, again supporting the validity of our expression analysis (Fig. 3B). In addition to trichomes, the petiole displayed strong promoter reporter activity. In the root, WRKY8 is prominently expressed in the vasculature and the emerging lateral roots (Fig. 3D). T-DNA insertion alleles lacking detectable transcripts were identified for both genes (Supplemental Fig S3), but for both genes no alterations in trichome phenotype were detected in homozygotes of any of these insertion alleles. Since some of the genes involved in trichome patternning have redundant functions, a double mutant was generated of wrky8 with ttk2 (wrky44) and in addition with ttk1, try, and cpc. Again, no effect of the wrky8 mutation on the described trichome phenotypes of the single mutants was observed (data not shown). Since expression of several other members of the WRKY transcription factor family was detected in trichomes (WRKY3 [AT2G03340], WRKY15 [AT2G23320], WRKY18 [AT4G31800], WRKY33 [AT2G38470], and WRKY40 [AT1G80840]), there might be other genes that can compensate for a loss of WRKY8 function.

In addition to At1g66460 and WRKY8, T-DNA mutant lines for another 45 trichome-specific genes were analyzed for alterations in trichome development. No obvious differences in morphology or trichome patternning were found after inspection with a dissecting microscope (Supplemental Table S3). However, neither the insertion sites nor the expression patterns were determined for the respective T-DNA lines, and we cannot unambiguously rule out the possibility that among the selected genes some are required for trichome development or physiology. However, wherever possible, two alleles of a given candidate gene were analyzed to make the discovery of a mutant phenotype most likely (a total of 78 T-DNA lines for 45 genes).

Mapping and Molecular Identification of NOK

In contrast to the cases described above, for one gene overrepresented in trichomes compared with leaves without trichomes, MYB106 (At1g01140), we could associate a mutant trichome phenotype with the loss of function of the respective gene. Besides a reverse genetics approach, a single cell transcriptome can be used in a forward genetics approach to identify the molecular nature of mutants in combination with map-based cloning. Over the last decade, many mutants with an altered trichome phenotype have been isolated. One not yet molecularly identified gene is defined by nok mutants, in which trichomes are overbranched and have a glassy appearance (Fig. 4B; Folkers et al., 1997).

By bulk segregant mapping (Lukowitz et al., 2000), the nok-gb allele was located on the upper arm of chromosome 3, north of the marker MDC16a IND1 (Fig. 4E). In an expanded mapping population, nok-gb was found to be distal to three markers, MDC16a IND1 (84 of 912 chromosomes recombinant), a marker on bacterial artificial chromosome (BAC) clone T11118 (26 of 1,168 chromosomes recombinant), and a marker on BAC clone F4P13 (1 of 1,168 chromosomes recombinant). The marker on F4P13 is located approximately 200 kb from the left telomere of chromosome 3. No markers distal to nok-gb were identified, consistent with a position very close to the telomere.

The mapped region containing the nok-gb mutation between the F4P13 marker and the telomere (TEL3N) comprised 84 genes. To identify candidate genes encoding NOK, we searched our transcriptome data for preferentially expressed genes in this chromosomal region. Only three genes were found to be more than 2-fold more highly expressed in trichomes than in leaves without trichomes (At3G01140, At3G01280, and At3G01350; Table I). Since several MYB transcription factors have been implicated in trichome development, MYB106 (At1g01140), located on the most telomere-proximal BAC, T4P13, was a key candidate for NOK. A genomic fragment of MYB106 was sequenced in nok-gb, and we found a transition from G to A at position 242 (with the A in the ATG of the genomic region being 2002; Ishida et al., 2007).
position +1), changing a highly conserved Cys of the first MYB repeat to a Tyr (Stracke et al., 2001). To corroborate that NOK is encoded by MYB106, we sequenced the genomic region of MYB106 in a second nok allele (nok-122). In nok-122, a G-to-A transition at position 435 was found, causing a mutation in the splice acceptor site between the second and third exons. A third allele, nok-9310-11, was generated by fast-neutron irradiation that is known to frequently lead to chromosomal rearrangements and could not be amplified by PCR. Finally, two T-DNA mutants (SALK_025449 and SALK_110059; Alonso et al., 2003) were ordered in which the T-DNA was annotated to be inserted in MYB106. Homozygous plants for both alleles showed the typical overbranched phenotype of the nok trichomes on rosette as well as cauline leaves (Supplemental Table S4; data not shown). The location of the T-DNAs was subsequently confirmed and is depicted in Figure 4F. Taken together, we conclude that NOK encodes the putative transcriptional regulator MYB106.

To further characterize NOK, a fusion of a 2.1-kb genomic DNA fragment upstream of MYB106 with a GUS gene was generated. This fragment conferred expression of the reporter gene in mature trichomes and, in addition, ubiquitous staining was found in emerging leaves (Fig. 4G). In flowers, strong GUS activity was found in the youngest part of pedicels, in petals, and on the outer surface of carpels (Fig. 4H).

NOK belongs to the MIXTA subfamily of MYB genes (Stracke et al., 2001), which are known from Antirrhinum to regulate growth of the petal cells, and an expression of NOK in Arabidopsis petals is consistent with this sequence conservation (Noda et al., 1994). However, analysis of nok petals by scanning electron microscopy did not show any alterations of the petal cell morphology (data not shown). Thus, MIXTA function might be redundantly represented in Arabidopsis, as there is a small subfamily of MYB transcription factors comprising in addition to MYB106 the genes MYB16 (At5g15310) and MYB17 (At3g61250). Alternatively, MIXTA-like genes might have adopted different functions in different species, and a first indication for this is that MIXTA in Antirrhinum promotes the outgrowth of epidermal cells on the petals while NOK in Arabidopsis appears to repress anisotropic growth (i.e. branching of trichomes).

Figure 4. Phenotypic characterization and cloning of the NOK gene. In the nok-gb mutant (B), the number of trichome branches is increased in comparison with the Col-0 wild type (A). The papillae normally seen on the surface of the wild-type trichomes (C) are completely missing in the nok-gb mutant (D). Bars = 50 μm. NOK was mapped to the upper arm of chromosome 3 (E), distal to marker F4P13. The numbers given for the markers indicate the amount of recombinant chromosomes. Four alleles of nok were isolated, and the positions of the mutations are indicated (F). In addition to the general staining of emerging leaves and mature trichomes (G), GUS activity was also found in carpels, petals, and stamens of a PROMYB106::GUS fusion (H).
DISCUSSION

Trichomes versus Other Arabidopsis Cell Types

Here, we present the cell type-specific expression profile of mature wild-type Arabidopsis trichomes and of two mutants, gl3 and try. Trichomes have been intensively used as a model for cell development. In addition, leaf hairs of different species are of great interest for applied research, for instance cotton fibers or peppermint trichomes. Together with the already existing knowledge about trichomes, our data now provide the basis for a systems biological understanding of this cell type. Additionally, the transcriptome information is a valuable resource for other research questions, for instance, the analysis of cell wall and wax biosynthesis.

We have identified here 5,461 genes (24% of the genes on the ATH-1 chip) that showed an expression level of more than 30 arbitrary expression units in trichomes. Similarly, 6,587 genes were found to be expressed in pollen (Pina et al., 2005), and comparable numbers of active genes can be extrapolated for guard and mesophyll cells, 1,309 and 1,479 genes of approximately 8,100 genes represented on the ATH 8k chip, respectively (Leonhardt et al., 2004). Thus, roughly one-quarter of all genes might cover most of the biological functions of a differentiated cell in Arabidopsis.

Of the 5,461 genes expressed above threshold levels, we identified 3,231 genes as differentially expressed in mature trichomes, including 1,115 genes that are essentially expressed exclusively in trichomes rather than other leaf tissues. However, some genes important for trichome development and function were not found in the gene set for several reasons. Unfortunately, at a technical level, a few genes are not represented on the Affymetrix GeneChip ATH-1, as is the case for the well-known trichome patterning gene GL3.

Other genes that have been shown to be required for proper trichome development might not be differentially expressed in trichomes, as for instance the WURM (WRM) gene, which regulates the actin cytoskeleton and displays an obvious loss-of-function phenotype in trichomes (Saedler et al., 2004). This behavior can be explained by regulation at the protein level in trichome or by genes functioning redundantly in other cells. Also, cytoskeletal function might be more limiting in trichomes than in other cells, so that even a slight reduction in a function might result in a detectable alteration of trichome differentiation while remaining sufficient in other cells. Indeed, while wrm and other mutants affecting the actin cytoskeleton only showed defects in trichomes under standard light growth conditions, growing the same mutants in the dark and by that treatment inducing additional expansion of hypocotyl cells (skotomorphogenesis) revealed a requirement of WRM also for rapidly expanding cells.

Importantly, the gene set identified here reflects only one, although the longest, stage of trichome development. Therefore, genes involved in pattern formation or early differentiation events might be only weakly expressed or not expressed at all in mature trichomes.

Specification of trichome cell fate shares a number of transcription factors and regulatory interactions with specification of root atrichoblasts. We could confirm here that many of the genes involved in pattern formation are active in both trichomes and atrichoblasts; in addition, 820 genes are expressed in both cell types. Other cell types in the root share only approximately half of the up-regulated genes with trichomes. This supports the idea that the transcription factor network governing the development of both trichomes and atrichoblasts drives the expression of many of the same genes in these cell types, although the morphology is completely different.

Relatively few differences were found between the transcriptomes of gl3 and try mutant trichomes in comparison with the wild type. One explanation for this is that both GL3 and TRY functions appear to be backed up by redundantly acting genes, such as ENHANCER OF GL3, ETC1, ETC2, ETC3/CPL3, and TCL1 (Zhang et al., 2003; Kirik et al., 2004a, 2004b; Simon et al., 2007; Wang et al., 2007; Tominaga et al., 2008). Alternatively, trichome differentiation might represent a robust program that is buffered against alterations during early patterning or changes of the endoreplication levels.

Arabidopsis Trichomes as Potential Model Cells to Study Cell Wall Biosynthesis

Remarkably, genes involved in cell wall formation were overrepresented in the transcriptome of mature trichomes presented here. Two genes encoding glucosyltransferases (MUR2 and MUR3) that were identified in a screen for Arabidopsis plants with altered monosaccharide composition of their cell wall show morphological defects only in the surface papillae of trichomes (Reiter et al., 1997; Vanzin et al., 2002; Madson et al., 2003), and one of these genes, MUR2, was found to be 2.64-fold up-regulated in trichomes.

Mutations in several other uncharacterized genes also cause glassy trichomes, such as RETSINA, CHARDONNAY, CHABLIS (Hulskamp et al., 1994), and UNDERDEVELOPED TRICHOMES1 (Haughn and Somerville, 1988), that might also be affected in cell wall biosynthesis. The loss of surface papillae is also responsible for the glassy appearance of the trichomes in the nok mutant that we characterize here molecularly. Since NOK encodes a MYB transcription factor, transcript profiling of the mutant trichomes might give insights into the interaction of genes involved in the formation of papillae. Therefore, trichomes seem to be sensitive indicators for defects in cell wall biosynthesis and, thus, might serve as a model system for further studies.

Similarly, the transcriptome of trichomes contains at least 41 genes that are potentially involved in cuticular wax biosynthesis. Mutations in five genes of this pathway have already been found to produce trichomes with an aberrant phenotype (Xia et al., 1997; Yephremov et al., 2007).

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Arabidopsis Trichomes in Comparison with Cotton Fibers

The generation of shoot epidermal hairs is very common in the plant kingdom (Uphof, 1962). In general, two major hair types can be distinguished: secreting trichomes with glandular heads, as found in peppermint, tomato (Solanum lycopersicum), alfalfa (Medicago sativa), and others; and nonglandular trichomes, as found in cotton and Arabidopsis. Secreting trichomes are usually multicellular, whereas nonglandular trichomes can be unicellular as in cotton and Arabidopsis or multicellular as in Antirrhinum majus. Whether all trichomes share a common developmental program has been unclear. For example, the bHLH transcription factor R is needed for anthocyanin production but not for trichome formation in maize (Zea mays). Its heterologous expression promotes anthocyanin synthesis in both tobacco (Nicotiana tabacum) and Arabidopsis but a massive generation of trichomes only in Arabidopsis (Lloyd et al., 1992).

Since trichomes are often of industrial interest, it would be desirable to have an easily accessible model system like Arabidopsis available to promote the understanding of trichome development and physiology in economically important species. The transcriptome comparison presented here indicates that Arabidopsis and cotton trichomes are indeed closely related. The similarity extends to likely regulators of cell fate and differentiation. For example, previous experiments have shown that a promoter active in cotton fibers can also drive expression in Arabidopsis trichomes (Wu and Liu, 2006), that the overexpression of the cotton GaMYB2 can induce the development of trichomes on the outer integuments of Arabidopsis seeds (Wang et al., 2004), and that a TTG1 homolog from cotton can complement the ttg1 mutation in Arabidopsis (Humphries et al., 2005). Conversely, at least three of the trichome patterning genes are also expressed on the integuments of Arabidopsis seeds (i.e. TTG1, TTG2, and GL2).

From our profiling approach, we found that AtMYB106, the homolog of the MIXTA-like MYB transcription factor GhMyb25 from cotton, is an important regulator of Arabidopsis trichome outgrowth, providing further evidence for the close similarity of the regulatory programs of these two types of trichomes.

A Function for MIXTA-Like Genes during Arabidopsis Trichome Development

Arabidopsis trichomes have been extensively studied, and major breakthroughs have been achieved in the understanding of pattern formation and the control of branch formation. However, the control of trichome cell outgrowth is still poorly understood.

The profiling data presented were used to assist map-based cloning of NOK. nok is the only known trichome mutant so far that shows increased branching without a concomitant increase in endoreplication levels (Folker et al., 1997). In addition, nok mutant trichomes are glassy and lack or display a reduced number of papillae in the surface. The exact biological function of these papillae still remains to be understood. While trichome branches are initiated at early stages before the completion of the endoreplication program, papillae are formed only later, after trichomes are three branched. This suggested that NOK function is required in maturing trichomes; indeed, NOK is among the 200 most strongly expressed genes in the mature trichome transcriptome.

NOK is encoded by MYB106 (At3g01140), which belongs to subgroup 9 of the R2R3 MYB transcription factors (Stracke et al., 2001), represented by its first characterized member, MIXTA from Antirrhinum majus. MIXTA-like MYB genes are required for the outgrowth of petal cells in the asterids Antirrhinum and Petunia (Avila et al., 1993; Noda et al., 1994; Baumann et al., 2007). Moreover, MIXTA genes can also affect the patterning of trichomes in asterids, and heterologous expression of AmMIXTA in tobacco promoted ectopic conical cells in leaves and trichomes in carpels (Glover et al., 1998). In contrast, heterologous expression of AmMIXTA in the rosid Arabidopsis has no effect on conical cell or trichome formation. Moreover, NOK in Arabidopsis appears to restrict directional cell expansion, suggesting that MIXTA genes might function in Arabidopsis as outgrowth repressors. This suggests that the different developmental programs of leaf trichomes include similar genes but that these genes diverged functionally since the separation of the asterids and rosids (Serna and Martin, 2006).

An important task for the future will be to identify target genes of MIXTA-like MYBs in Antirrhinum in comparison with Arabidopsis. A first hint of the downstream genes might come from the glassy appearance of nok mutants, a phenotype shared by mur3 and mur2 mutants, which are deficient in fucosyltransferase encoded by FUT1 (Vanzin et al., 2002; Madson et al., 2003). FUT1 was up-regulated in trichomes; therefore, it is tempting to speculate that FUT1 and other cell wall-related genes might be targets of MYB106. Other molecularly uncharacterized mutants that lack papillae, including underdeveloped trichomes1, might identify other targets of MYB106 (Haughn and Somerville, 1988).

Profiling of nok mutants as well as NOK-overexpressing plants might now be an important next step to gain deeper insight into the transcriptional network controlling downstream events of cell growth. Furthermore, since NOK was not found to be expressed in roots, it might represent one of the regulators that cause the phenotypic divergence between trichomes and atriarchoblasts, despite their similar regulators of patterning.
MATERIALS AND METHODS

Plant Material, Growth Conditions, and Plant Transformation

Different Arabidopsis (Arabidopsis thaliana) accessions show variability in trichome development (Hauser et al., 2001). To avoid the possibility that accession-specific properties would disturb the transcript profiling, Arabidopsis accession Col-0 was used in comparison with the mutant try-JC in the Col-0 genetic background (Larkin et al., 1999). To obtain a g13 mutant in Col-0, we isolated a new g13 allele from the Gabi-Kat collection (545D05; Rossos et al., 2003), designated g13-3 (Supplemental Fig. S3). In addition to the nok-122 reference allele (Folkers et al., 1997), four additional nok alleles have been obtained. The nok-gb allele originated in a Col-0 ethyl methanesulfonate-mutagenized M2 population and was backcrossed three times to Col-0 before selfing and selecting the mutant line used. The nok-9310-11 allele originated in an M2 screen of fast-neutron mutagenized seeds (Luo and Oppenheimer, 2000; MYB06 At3g01400, J724, 5–GGGCCCCCACTCATGTCATGTC-3’, amplified a 1.4-kb fragment; MDC16a IND1, 5–CCCCAATCCGTATGTCATGTC-3’, amplified a 2.0-kb fragment; WRKY8 At1g46530, J710, 5–GGGCCCACTCATGTCATGTC-3’, and J709, 5–GGGCCCCCACTCATGTCATGTC-3’, amplified a 2.1-kb fragment; MYB06 At3g01400, J724, 5–GGGCCCCCACTCATGTCATGTC-3’, and J725, 5–CCCCAATCCGTATGTCATGTC-3’, amplified a 1.4-kb fragment (Ascl, Xhol, and Sall restriction sites are underlined). After PCR with MBI Fermentas’s Long PCR Mix on chromosomeal DNA, the PCR products were purified (Nucleo Spin Extract II; Macherey-Nagel) and digested with Ascl and Xhol and ligated in Ascl- and Xhol-digested PAPAMPtgvep. After verification of the resulting constructs by sequencing, the GUS gene was introduced via a Gateway LR reaction. Constructs were transformed into Arabidopsis as described (Jasby et al., 2006). The expression of the GUS protein was visualized using methods described previously (Weinl et al., 2005). For every construct, 24 T1 lines were stained and at least 12 showed the presented expression pattern.

Preparation of RNA from Trichomes and Leaves

For the T samples, primary rosette leaves were attached to slides with double-sided tape and frozen on dry ice. Trichomes were clipped off at their stalk, leaving their base on the leaf in order to avoid contamination with adjacent epidermal or mesophyll cells, using extra-fine forceps and immediately transferred to RLT buffer (Qiagen). For the LwOT samples, trichomes were removed from leaves, slides were frozen on blocks of dry ice, and the trichomes were removed with a small brush. Leaves were removed from the slides, washed briefly in water, and transferred to RLT buffer. RNA was isolated using the RNeasy Micro kit (Qiagen). cDNA was quantified with the Agilent Bioanalyzer and Nano6000 chips. Fifty to 100 ng of total RNA was used as starting material for the RNA amplifications.

Amplification of RNA, Labeling, and Hybridizing to Affymetrix ATH-1 GeneChips

RNA was amplified according to the Affymetrix Eukaryotic Protocol (Col-0) or the Arcturus RiboAmp OA protocol (g13-2 or try-JC). Amplification of the RNA was monitored with the Agilent Bioanalyzer. Approximately 5 μg of the amplified RNA was labeled with the Affymetrix GeneChip Expression Analysis 3’ Amplification Reagents for IVT Labeling kit and hybridized to Affymetrix ATH-1 GeneChips at the MTTBI Affymetrix Unit at the Medical Department of the University of Cologne. In order to conduct a statistically appropriate analysis of the expression profiles, we performed microarray hybridizations in biological triplicates for trichomes (Col-0, g13-2, and try-JC) and LwOT.

GeneChip Data Analysis

All statistical analyses of the microarray data were conducted in R (www.r-project.org) employing BioConductor (www.bioconductor.org) facilities. Probe sets of each of the triplicates were summarized with the MAS5.0 algorithm. High variance between replicates may have several sources, including biological variability and technical causes. Probe sets affected by technical variability were removed (LwoT control group) employing a LOESS smoother operating on logarithmic M-A scale (Cleveland, 1979).

The cutoff for the believable baseline signal level was set to 30 arbitrary expression units (linear scale) to ascertain that genes known to be expressed in trichomes (Supplemental Table S5) remain in the filtered set of 5,461 probe sets. Of these, 3,231 genes are induced in trichomes compared with LwOT. Probe sets differentially expressed between LwOT and Col-0 trichomes are tested by a Bayesian regularized t test (Baldi and Long, 2001). The flow of the analysis is depicted in Supplemental Fig. S4.

Construction of PROMOTER:GUS Fusions

In order to verify the results, fusions of putative promoter regions of candidate genes were amplified and fused to the GLU gene. Genes, primers, and sizes of the amplified regions were as follows: putative protein kinase At5g66460 J685, 5–GGGCCCACTCATGTCATGTC-3’, amplified a 2.0-kb fragment; WRKY8 At1g46530, J710, 5–GGGCCCACTCATGTCATGTC-3’, and J709, 5–GGGCCCCCACTCATGTCATGTC-3’, amplified a 2.1-kb fragment; MYB06 At3g01400, J724, 5–GGGCCCCCACTCATGTCATGTC-3’, and J725, 5–CCCCAATCCGTATGTCATGTC-3’, amplified a 1.4-kb fragment (Ascl, Xhol, and Sall restriction sites are underlined). After PCR with MBI Fermentas’s Long PCR Mix on chromosomeal DNA, the PCR products were purified (Nucleo Spin Extract II; Macherey-Nagel) and digested with Ascl and Xhol and ligated in Ascl- and Xhol-digested PAPAMPtgvep. After verification of the resulting constructs by sequencing, the GUS gene was introduced via a Gateway LR reaction. Constructs were transformed into Arabidopsis as described (Jasby et al., 2006). The expression of the GUS protein was visualized using methods described previously (Weinl et al., 2005). For every construct, 24 T1 lines were stained and at least 12 showed the presented expression pattern.

Genotyping and Expression Analysis of Candidate Genes

Genotyping of T-DNA lines was carried out by amplification of a PCR product spanning the insertion site and a PCR product between a T-DNA-specific primer and one of the gene-specific primers. A homozygous mutant is characterized by the lack of the PCR product corresponding to the wild-type allele and a PCR product corresponding to the mutant allele. For WRKY8, primers J708 (5’-TGGCCATCATCATAACATC-3’) and J811 (5’-ATAAATATATATCAAGGCCTCTTGGTTG-3’) were used to amplify a 0.7-kb wild-type fragment. The mutant allele was detected with primers 8409 (5’-ATAATGACATCATCATAACATC-3’) and J811 and yielded a 0.5-kb fragment. For AT1g66460, primers J817 (5’-ATCCTCCCTGATCCCTCTCCTATGTCATGTC-3’) and J815 (5’-ATCCTCCTGATCCCTCTCCTATGTCATGTC-3’) amplified a 1.4-kb fragment for the wild-type allele. The mutant allele in GABI-Kat line 432D04 was amplified with primer J814 and primer 8409 and gave a 0.5-kb band, and the mutant allele in GABI-Kat line 653F03 was amplified using the same primer combination but yielded a 1.37-kb PCR product.

Expressed in the mRNA was tested by amplification of a cDNA fragment spanning the insertion sites of the T-DNA. For WRKY8, primers J811 and J816 (5’-ATGACTAAGGACGAAGTCTGATCCTCG-3’) were used, yielding a 0.47-kb fragment; for AT1g66460, primers J814 and J815 were used to amplify a 0.77-kb cDNA fragment.

Mapping of nok-gb

The mapping population was generated by crossing the Col-0-derived nok-gb allele with Landsberg erecta. The nok-gb allele was initially mapped via bulk segregant analysis to the marker MDC16a IND1 as described by Lukowitz et al. (2000). MDC16a IND1 is located on the same BAC clone as the nga162 marker used by Lukowitz et al. (2000). MDC16a IND1 is located on the same BAC clone as the nga162 marker used by Lukowitz et al. (2000). MDC16a IND1 is located on the same BAC clone as the nga162 marker used by Lukowitz et al. (2000). MDC16a IND1 is located on the same BAC clone as the nga162 marker used by Lukowitz et al. (2000). MDC16a IND1 is located on the same BAC clone as the nga162 marker used by Lukowitz et al. (2000).

Microscopy

Cryo-scanning electron microscopy was performed as described previously (Rumbolz et al., 1999).

The CEL files and the combined normalized data file are available at ArrayExpress with the accession number E-ATMM-33. NOK (MYB106) has the Arabidopsis Genome Initiative code At3g01100.
SUPPLEMENTAL DATA

The following materials are available in the online version of this article.

SUPPLEMENTAL Figure S1. GO analysis of the 250 genes with the lowest expression in trichomes in comparison with leaves without trichomes.

SUPPLEMENTAL Figure S2. Selected biosynthetic pathways up-regulated in trichomes as detected by the AraCyc tool at The Arabidopsis Information Resource (release 4.0; www.arabidopsis.org/biocyc/index.jsp).

SUPPLEMENTAL Figure S3. Genotyping and expression analysis of At1g66460 and wrky8 T-DNA lines.

SUPPLEMENTAL Figure S4. Flow chart explaining the steps of bioinformatic analysis of the Affymetrix chip experiments.

SUPPLEMENTAL Table S1. A. List of all 3,231 genes found to be differentially more highly expressed between trichomes (T) compared with leaves whose trichomes were removed (Lw0T), and the respective expression values found in gl3-3 and tyr-JC trichomes. B, Table containing all genes down-regulated in mature trichomes in comparison with Lw0T.

SUPPLEMENTAL Table S2. List of genes that are up-regulated in both Arabidopsis trichomes and root atrichoblasts (A), stele (B), lateral root cap (C), cortex (D), and endodermis (E).

SUPPLEMENTAL Table S3. List of T-DNA lines of candidate genes from up-regulated genes in Arabidopsis trichomes.

SUPPLEMENTAL Table S4. Trichome branch points in the wild type and different nok mutant alleles.

SUPPLEMENTAL Table S5. Genes with known expression/mutant phenotypes in trichomes used to define the cutoff for scoring a gene as expressed.

SUPPLEMENTAL Table S6. Selected biosynthetic pathways expressed in mature Arabidopsis trichomes.

SUPPLEMENTAL Table S7. The 10% most up-regulated genes of mature Arabidopsis trichomes that also show expression in root atrichoblasts.

SUPPLEMENTAL Table S8. Comparison of cotton fiber gene expression with Arabidopsis trichomes.

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