Transcriptional profiling of mature Arabidopsis trichomes reveals that NOECK encodes the MIXTA-like transcriptional regulator MYB106

Transcriptional Profiling of Mature Arabidopsis Trichomes Reveals That *NOECK* Encodes the MIXTA-Like Transcriptional Regulator MYB106[1][C][W][OA]

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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 *triptychon*, 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 in-

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stance, the aromatic substances in peppermint (*Mentha piperita*) and basil (*Ocimum basilicum*), including phenylpropenes and terpenoids, are synthesized in glandular trichomes (Lange et al., 2000; Iijima et al., 2004). In addition, trichomes are sites of detoxification (Gutierrez-Alcala et al., 2000) and are used by the plant as storage for heavy metals (Dominguez-Solis et al., 2004). The fibers of cotton (*Gossypium hirsutum*), of major interest to the textile industry, are also trichomes. Fibers are formed on the seed coat of *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 *glabra3* (*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 *triptychon* (*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 *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 *cdka;1* mutants, in which the central regulatory CDK, CDKA;1, 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 *noeck* (*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, *gl3* and *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 *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; Nawy 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 (LwoT sample) 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-

malized 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 30 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 gluconeogenesis 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, *mur2* 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 *ARABINOGALACTAN 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, *P* value for the comparison of the mean of three trichome chips (mT) with the mean of three leaves without trichome chips (mLwoT); FC, fold change; m*gl3-3*, the mean of three *gl3-3* trichome chips; m*try-JC*, the mean of three *try-JC* trichome chips.

pVAL	FC mT/mLwoT	Gene Title	Arabidopsis Genome Initiative Code	FC m*gl3-3*/mT	FC m*try-JC*/mT
0.07	109.20	Expressed protein	AT3G18170	1.05	1.68
0.03	99.37	GLABRA2	AT1G79840	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	AT3G09520	0.63	1.94
0.03	41.03	Hairpin-responsive protein, putative (HIN1)	AT5G06330	0.49	1.14
0.00	40.37	Phospho*enol*pyruvate carboxykinase, putative	AT4G37870	0.31	0.96
0.03	36.27	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	Strictosidine 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	Strictosidine 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 (DGAT)	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	WRKY8	AT5G46350	0.96	1.95
0.05	21.05	Expressed protein	AT1G13340	1.20	1.39
0.00	20.36	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 b_5, 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	L-Lactate dehydrogenase, putative	AT4G17260	0.30	0.60
0.10	16.48	Peroxidase, putative/peroxidase 33	AT3G49120 AT3G49110	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	AT2G34850	0.49	1.51
0.04	14.43	Integral membrane transporter family protein	AT1G64890	0.84	1.31
0.04	13.98	Expressed protein	AT4G22270	0.51	1.66
0.04	13.80	SEC14 cytosolic factor family protein/phosphoglyceride transfer family protein	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 I. (*Continued from previous page.*)

pVAL	FC mT/mLwoT	Gene Title	Arabidopsis Genome Initiative Code	FC m*gl3-3*/mT	FC m*try-JC*/mT
0.02	12.22	Dehydrin xero2 (XERO2)/low-temperature-induced protein LTI30 (LTI30)	AT3G50970	0.48	1.13
0.04	12.21	Fasciclin-like arabinogalactan 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-CI)/17.8-kD class I heat shock protein (HSP17.8-CI)	AT1G59860 AT1G07400	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 POZ protein-related	AT3G48360	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	AT2G46410	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	Ca^{2+}-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	AT1G59970	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 AT5G10700	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 I 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

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Table I. (*Continued from previous page.*)

pVAL	FC mT/mLwoT	Gene Title	Arabidopsis Genome Initiative Code	FC m*gl3-3*/mT	FC m*try-JC*/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	AT5G65300	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	AT5G44130	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	Pectinacetylesterase, putative	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 (RCI2A)/low-temperature- and salt-responsive protein (LTI6A)	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	ABI3-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)	AT1G27450	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	AT4G13770	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	Glc 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	Lipoxygenase (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.*)

Table I. (*Continued from previous page.*)

pVAL	FC mT/mLwoT	Gene Title	Arabidopsis Genome Initiative Code	FC m*gl3-3*/mT	FC m*try-JC*/mT
0.13	5.34	Low-temperature-responsive protein 78 (LTI78)/desiccation-responsive protein 29A (RD29A)	AT5G52310	0.35	1.52
0.00	5.28	Histone H2A.F/Z	AT3G54560	0.78	0.61
0.01	5.27	Heavy metal-associated domain-containing protein/copper chaperone (CCH)-related	AT4G35060	0.76	1.90
0.01	5.27	Expressed protein	AT3G60520	0.41	1.17
0.00	5.24	LIM domain-containing protein	AT2G39900	0.56	0.28
0.07	5.21	Microtubule-associated EB1 family protein	AT5G62500	0.47	0.95

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 PRO_{GL2}:*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-

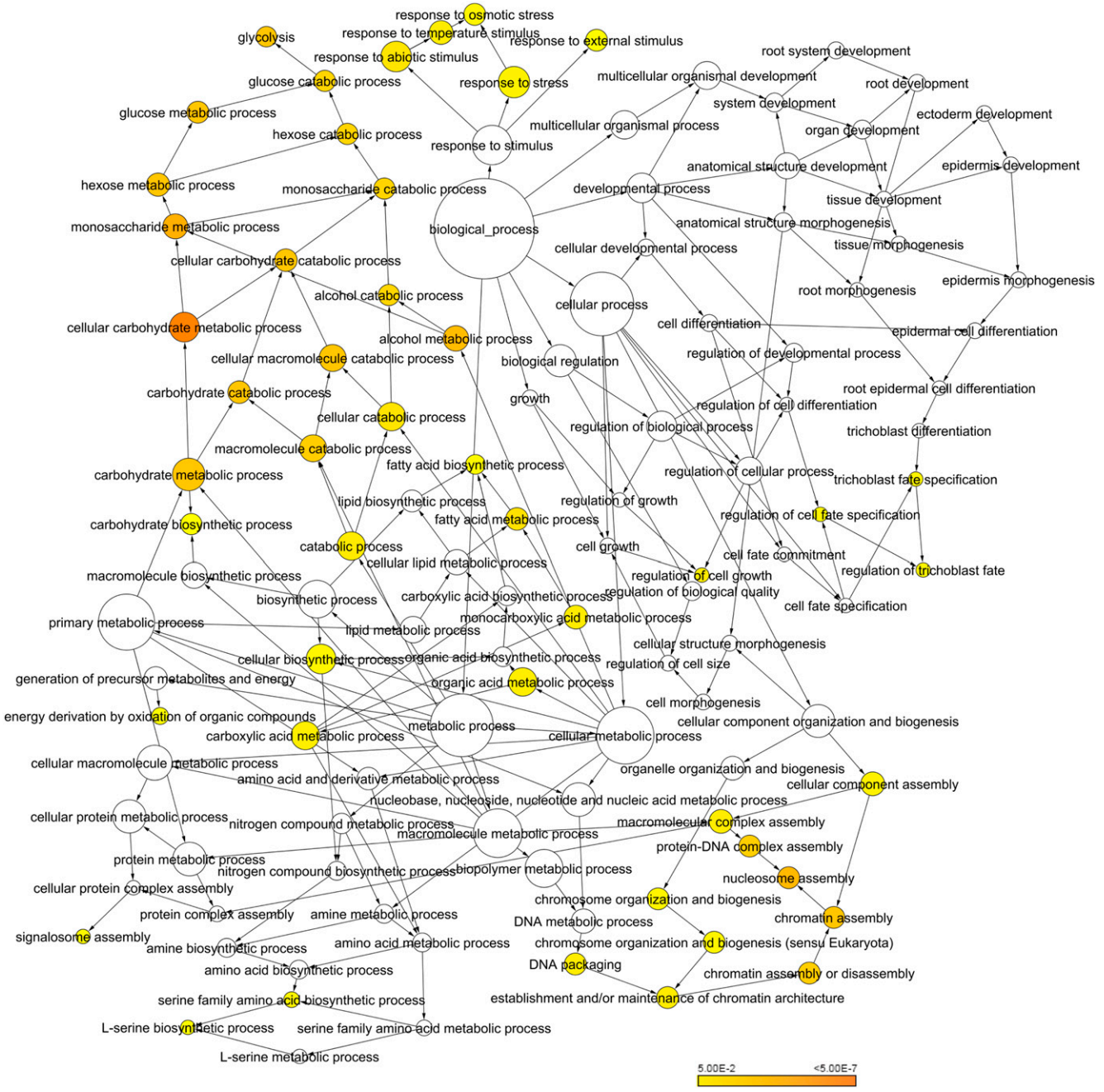

Figure 1. GO analysis of genes that are 2-fold more highly expressed in trichomes than in leaves without trichomes. The size of a node within the network is proportional to the number of genes in the respective GO category. The level of significance of an overrepresented GO category is indicated by the 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.]

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 HOMEODOMAIN GLABROUS (HDG; Nakamura et al., 2006). Among the *HDG*s, *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-

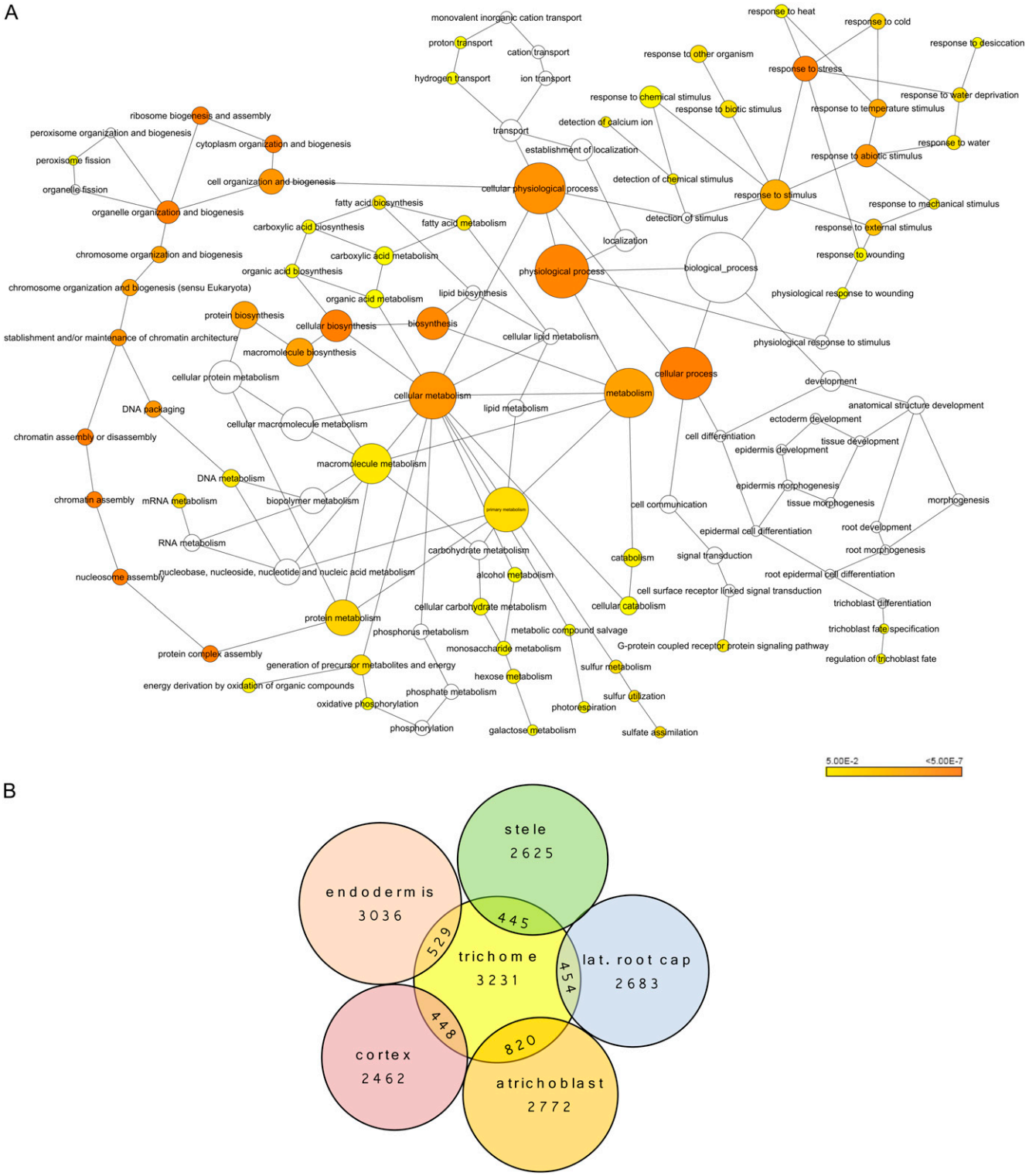

Figure 2. Comparison of expression profiles between trichomes and atrichoblasts. A, GO analysis of genes expressed in both trichomes and atrichoblasts. For annotation, see Figure 1. B, Venn diagram showing the overlap of expression of genes in trichomes and different cell types of the root. Please note 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 m*gl3-3*/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	AT4G00950	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 (AIP1)	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 I 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 I heat shock protein (HSP17.6A-CI)/17.8-kD class I heat shock protein (HSP17.8-CI)	AT1G59860 /// AT1G07400	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 m*gl3-3*/mT
The 25 most up-regulated genes in *try-JC* trichomes		
Arabinogalactan protein (AGP4)	AT5G10430	3.60
Peroxisomal protein PEX19 family protein	AT3G03490	3.46
Peroxidase, putative/peroxidase 33 (PER33; P33; 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	AT3G52420	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 (PLC1)	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	AT1G51980	0.16
Geranylgeranyl pyrophosphate synthase, putative	AT4G38460	0.15
Senescence-associated protein (SEN1)	AT4G35770	0.15
Elongation factor 1B α-subunit 1 (eEF1Bα1)	AT5G12110	0.15
Expressed protein	AT3G15630	0.15
Expressed protein	AT2G15890	0.15
17.6-kD class I small heat shock protein (HSP17.6C-CI; amino acids 1–156)	AT1G53540	0.14
Pyruvate kinase, putative	AT5G63680	0.14
Alkyl hydroperoxide reductase/thiol-specific antioxidant (AhpC/TSA)/mal allergen family protein	AT3G06050	0.13
Hevein-like protein (HEL)	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	AT5G08500	0.12
Man-6-P reductase (NADPH-dependent), putative/Man-6-P reductase (NADPH-dependent), putative	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)	AT1G59860/ AT1G07400	0.10
β-Ketoacyl-CoA synthase family protein	AT1G07720	0.09
Transport protein particle (TRAPP) component Bet3 family protein	AT3G05000	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)	AT1G53910	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 *cpc-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 (Kirik 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 underreplicated *gl3* trichomes (0.23-fold) and slightly more strongly expressed in the overreplictated *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* (*SMR1*), 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 overrepresented 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 *GhMyb25*, 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 stomata 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; Ishida et al., 2007). 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 patterning have redundant functions, a double mutant was generated of *wrky8* with *ttg2* (*wrky44*) and in addition with *ttg1*, *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.

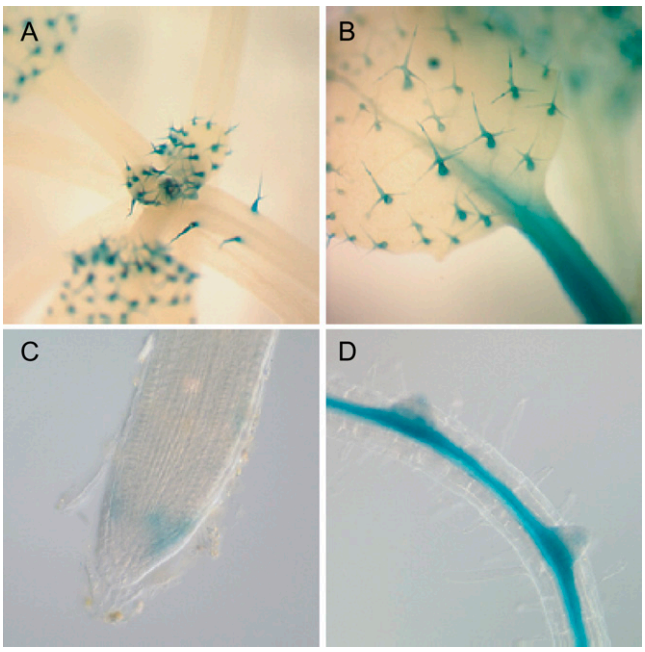

Figure 3. GUS staining of promoter reporter lines of identified trichome-specific genes. A fusion of the promoter of a putative protein kinase (*At1g66460*) to *GUS* showed expression in trichomes (A) and patches of epidermal cells at the root tip (C). Expression of a PRO_{WRKY8}:*GUS* fusion was detected in trichomes (B) and the vascular tissue of the root (D).

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 patterning 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* (*At3g01140*), 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 T11I18 (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* (*At3g01140*), 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

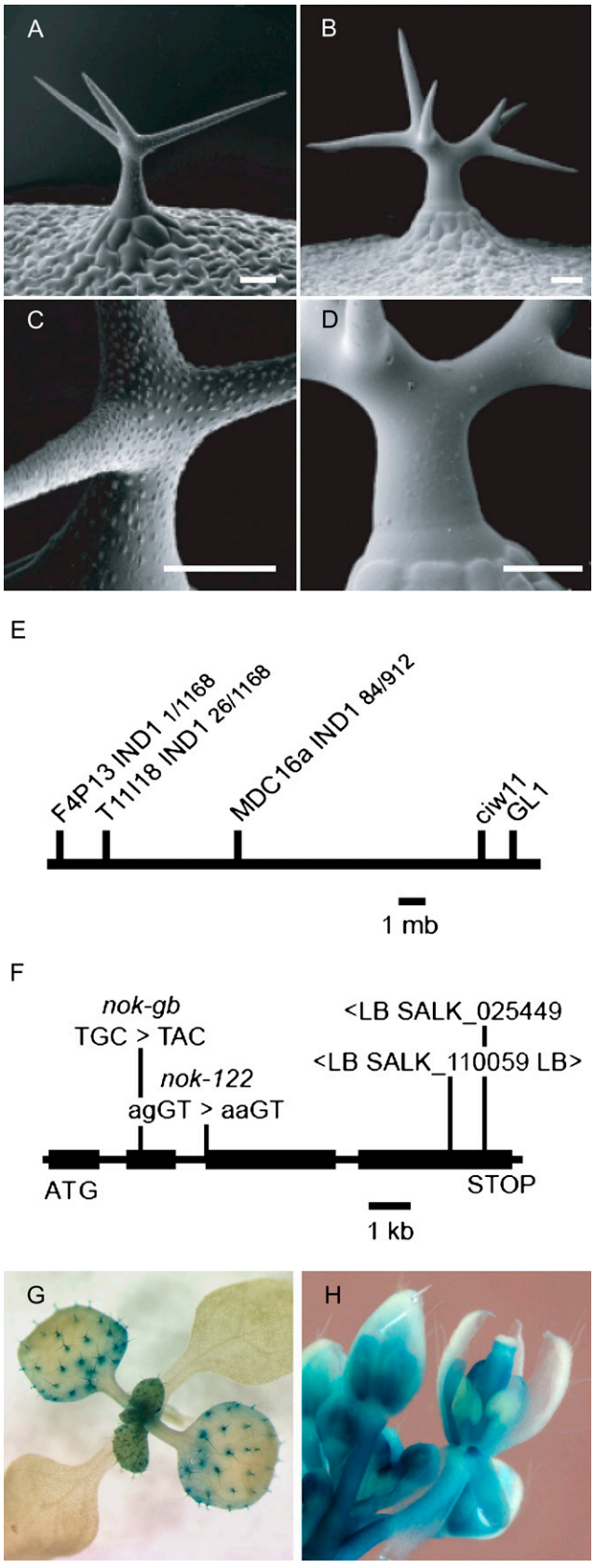

Figure 4. Phenotypic characterization and cloning of the *NOK* gene. In the *nok-gb* mutant (B), the number of trichome branches is increased in comparison with the Col-0 wild type (A). The papillae normally seen on the surface of the wild-type trichomes (C) are completely missing in the *nok-gb* mutant (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 PRO_{MYB106}:*GUS* fusion (H).

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).

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 *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.,

1999; Wellesen et al., 2001; Kurata et al., 2003; Zheng et al., 2005). Thus, the analysis of trichomes on T-DNA insertion lines for the remaining 36 genes might provide an easily accessible test system for involvement in cuticular wax production versus a function in other branches of the lipid biosynthesis pathway.

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*. 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 (Folkers 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 atrichoblasts, 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 *gl3* mutant in Col-0, we isolated a new *gl3* allele from the Gabi-Kat collection (GK 545D05; Rosso et al., 2003), designated *gl3-3* (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, 1999) and was a gift from Dr. David Oppenheimer (University of Florida). Both *nok-gb* and *nok-9310-11* fail to complement the *nok-122* reference allele in the F1 of intercrosses, and *nok-gb* mapped on the same arm of chromosome 3 (left arm) as *nok-122* in preliminary mapping studies. T-DNA insertions in *NOK* were obtained from the SALK collection (SALK_025449 and SALK_110059). Seeds were sown on soil, and pots were stored at 4°C for 3 d. Plants for profiling experiments were grown in a climate chamber for 13 d in a 10-h-light/14-h-dark cycle at 23°C.

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). RNA 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 using the Arcturus RiboAmp OA kit (*gl3-2* 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 3′-Amplification Reagents for IVT Labeling kit and hybridized to Affymetrix ATH-1 GeneChips at the MTBTI 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, *gl3-2*, 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 MAS-5.0 algorithm. High variance between replicates may have several sources, including biological variability and technical causes. Probe sets affected by these are poor with regard to their information content. Hence, probe sets with a difference between the Col-0 trichome samples higher than 2-fold were excluded from the analysis. The MAS-5.0 summaries of the remaining 18,459 probe sets of the Col-0 trichome GeneChips and of the *gl3-3* and *try-JC* mutant triplicates were normalized against the mean of the leaves whose trichomes were removed (LwoT control group) employing a LOESS smoother operating on logarithmic M-A scale (Cleveland, 1979).

The cutoff for the reasonable 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 *GUS* gene. Genes, primers, and sizes of the amplified regions were as follows: putative protein kinase *At1g66460*, J685, 5′-GGGGCGCGCCAAGTTATTTGTATTGTATATAATTTTAGC-3′, and J686, 5′-CCCTCGAGCAAATGAGTTTTTATTTCTAGATTGTTGTC-3′, amplified a 2.0-kb fragment; *WRKY8 At5g46350*, J710, 5′-GGGGCGCGCCAATTAAGCTTTAATTATCTTCCTAATAAC-3′, and J709, 5′-GGGGTCGACGAAGAACAAAGAGAAAAAACTTAAAC-3′, amplified a 2.1-kb fragment; *MYB106 At3g01140*, J724, 5′-GGGGCGCGCCATGCGTGTGACTGACTTGTTGTTC-3′, and J725, 5′-CCCTCGAGTGTTTTCCCACACAAGTTATTAGTC-3′, amplified a 1.4-kb fragment (*Asc*I, *Xho*I, and *Sal*I restriction sites are underlined). After PCR with MBI Fermenta's Long PCR Mix on chromosomal DNA, the PCR products were purified (Nucleo Spin Extract II; Macherey-Nagel) and digested with *Asc*I and *Xho*I or *Sal*I and ligated in *Asc*I- and *Xho*I-digested pAMPATgwy. 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 (Jakoby 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′-TGCTCACATTCACATATACATATCATTC-3′) and J811 (5′-ATATTATATCATCAAGGCTCTTGTTTG-3′) were used to amplify a 0.7-kb wild-type fragment. The mutant allele was detected with primers 8409 (5′-ATATTGACCATCATACTCATTGC-3′) and J811 and yielded a 0.5-kb fragment. For *AT1G66460*, primers J814 (5′-ATTCTTTGATCCGATTCAGTTACC-3′) and J815 (5′-ACTCCTCTGTAGCTTCTATCCCTCTAT-3′) amplified a 1.43-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.

Expression of the mRNAs was tested by amplification of a cDNA fragment spanning the insertion site of the T-DNA. For *WRKY8*, primers J811 and J816 (5′-ATGACTAAGACCGAAGTTGATCATCTCG-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) but gave more reliable results in our hands. The primers used to amplify the MDC16a IND1 insertion/deletion marker are 5′-GGAGTGGCCTCGTGTAGAGAA-3′ and 5′-CGCTACTTCCACTTAGACTCATCATC-3′. The other markers used in the mapping were derived from the Cereon database; the primers were as follows: T11I18, 5′-TCCTCCAAATCCAATGCCTT-3′ and 5′-AAATTGACCCCTACCCGCTC-3′; F4P13 IND1, 5′-TAACGCAGTGTACAATCAAGACGAGG-3′ and 5′-CCCGAGACGAATCCAAGACA-3′.

Microscopy

Cryoscanning 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-ATMX-33. *NOK* (*MYB106*) has the Arabidopsis Genome Initiative code At3g01140.

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 (LwoT), and the respective expression values found in *gl3-3* and *try-JC* trichomes. B, Table containing all genes down-regulated in mature trichomes in comparison with LwoT.

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 trichomes that also show expression in root atrichoblasts.

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

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LITERATURE CITED

Alonso JM, Stepanova AN, Leisse TJ, Kim CJ, Chen H, Shinn P, Stevenson DK, Zimmerman J, Barajas P, Cheuk R, et al (2003) Genome-wide insertional mutagenesis of Arabidopsis thaliana. Science **301:** 653–657

Arpat AB, Waugh M, Sullivan JP, Gonzales M, Frisch D, Main D, Wood T, Leslie A, Wing RA, Wilkins TA (2004) Functional genomics of cell elongation in developing cotton fibers. Plant Mol Biol **54:** 911–929

Avila J, Nieto C, Canas L, Benito MJ, Paz-Ares J (1993) Petunia hybrida genes related to the maize regulatory C1 gene and to animal myb proto-oncogenes. Plant J **3:** 553–562

Aziz N, Paiva NL, May GD, Dixon RA (2005) Transcriptome analysis of alfalfa glandular trichomes. Planta **221:** 28–38

Baldi P, Long AD (2001) A Bayesian framework for the analysis of microarray expression data: regularized t-test and statistical inferences of gene changes. Bioinformatics **17:** 509–519

Baumann K, Perez-Rodriguez M, Bradley D, Venail J, Bailey P, Jin H, Koes R, Roberts K, Martin C (2007) Control of cell and petal morphogenesis by R2R3 MYB transcription factors. Development **134:** 1691–1701

Becker JD, Boavida LC, Carneiro J, Haury M, Feijo JA (2003) Transcriptional profiling of Arabidopsis tissues reveals the unique characteristics of the pollen transcriptome. Plant Physiol **133:** 713–725

Beisson F, Koo AJ, Ruuska S, Schwender J, Pollard M, Thelen JJ, Paddock T, Salas JJ, Savage L, Milcamps A, et al (2003) Arabidopsis genes involved in acyl lipid metabolism: a 2003 census of the candidates, a study of the distribution of expressed sequence tags in organs, and a Web-based database. Plant Physiol **132:** 681–697

Bernhardt C, Lee MM, Gonzalez A, Zhang F, Lloyd A, Schiefelbein J (2003) The bHLH genes GLABRA3 (GL3) and ENHANCER OF GLABRA3 (EGL3) specify epidermal cell fate in the Arabidopsis root. Development **130:** 6431–6439

Birnbaum K, Shasha DE, Wang JY, Jung JW, Lambert GM, Galbraith DW, Benfey PN (2003) A gene expression map of the Arabidopsis root. Science **302:** 1956–1960

Bowman JL (1994) Arabidopsis: An Atlas of Morphology and Development. Springer Verlag, New York

Brady SM, Orlando DA, Lee JY, Wang JY, Koch J, Dinneny JR, Mace D, Ohler U, Benfey PN (2007) A high-resolution root spatiotemporal map reveals dominant expression patterns. Science **318:** 801–806

Carpita N, Tierney M, Campbell M (2001) Molecular biology of the plant cell wall: searching for the genes that define structure, architecture and dynamics. Plant Mol Biol **47:** 1–5

Churchman ML, Brown ML, Kato N, Kirik V, Hulskamp M, Inze D, De Veylder L, Walker JD, Zheng Z, Oppenheimer DG, et al (2006) SIAMESE, a plant-specific cell cycle regulator, controls endoreplication onset in *Arabidopsis thaliana*. Plant Cell **18:** 3145–3157

Cleveland WS (1979) Robust locally weighted regression and smoothing scatterplots. J Am Stat Assoc **74:** 829–836

Dissmeyer N, Nowack MK, Pusch S, Stals H, Inze D, Grini PE, Schnittger A (2007) T-loop phosphorylation of *Arabidopsis* CDKA;1 is required for its function and can be partially substituted by an aspartate residue. Plant Cell **19:** 972–985

Dominguez-Solis JR, Lopez-Martin MC, Ager FJ, Ynsa MD, Romero LC, Gotor C (2004) Increased cysteine availability is essential for cadmium tolerance and accumulation in Arabidopsis thaliana. Plant Biotechnol J **2:** 469–476

Folkers U, Berger J, Hulskamp M (1997) Cell morphogenesis of trichomes in Arabidopsis: differential control of primary and secondary branching by branch initiation regulators and cell growth. Development **124:** 3779–3786

Glover BJ, Perez-Rodriguez M, Martin C (1998) Development of several epidermal cell types can be specified by the same MYB-related plant transcription factor. Development **125:** 3497–3508

Gutierrez-Alcala G, Gotor C, Meyer AJ, Fricker M, Vega JM, Romero LC (2000) Glutathione biosynthesis in Arabidopsis trichome cells. Proc Natl Acad Sci USA **97:** 11108–11113

Haughn GW, Somerville CR (1988) Genetic control of morphogenesis in Arabidopsis. Dev Genet **9:** 73–89

Hauser MT, Harr B, Schlotterer C (2001) Trichome distribution in Arabidopsis thaliana and its close relative Arabidopsis lyrata: molecular analysis of the candidate gene GLABROUS1. Mol Biol Evol **18:** 1754–1763

Honys D, Twell D (2003) Comparative analysis of the Arabidopsis pollen transcriptome. Plant Physiol **132:** 640–652

Hulskamp M (2000) How plants split hairs. Curr Biol **10:** R308–310

Hulskamp M, Misra S, Jurgens G (1994) Genetic dissection of trichome cell development in Arabidopsis. Cell **76:** 555–566

Humphries JA, Walker AR, Timmis JN, Orford SJ (2005) Two WD-repeat genes from cotton are functional homologues of the Arabidopsis thaliana TRANSPARENT TESTA GLABRA1 (TTG1) gene. Plant Mol Biol **57:** 67–81

Iijima Y, Davidovich-Rikanati R, Fridman E, Gang DR, Bar E, Lewinsohn E, Pichersky E (2004) The biochemical and molecular basis for the divergent patterns in the biosynthesis of terpenes and phenylpropenes in the peltate glands of three cultivars of basil. Plant Physiol **136:** 3724–3736

Ishida T, Hattori S, Sano R, Inoue K, Shirano Y, Hayashi H, Shibata D, Sato S, Kato T, Tabata S, et al (2007) *Arabidopsis* TRANSPARENT TESTA

GLABRA2 is directly regulated by R2R3 MYB transcription factors and is involved in regulation of GLABRA2 transcription in epidermal differentiation. Plant Cell **19:** 2531–2543

Jakoby MJ, Weinl C, Pusch S, Kuijt SJ, Merkle T, Dissmeyer N, Schnittger A (2006) Analysis of the subcellular localization, function, and proteolytic control of the Arabidopsis cyclin-dependent kinase inhibitor ICK1/KRP1. Plant Physiol **141:** 1293–1305

Johnson CS, Kolevski B, Smyth DR (2002) TRANSPARENT TESTA GLABRA2, a trichome and seed coat development gene of *Arabidopsis*, encodes a WRKY transcription factor. Plant Cell **14:** 1359–1375

Jones MA, Raymond MJ, Smirnoff N (2006) Analysis of the root-hair morphogenesis transcriptome reveals the molecular identity of six genes with roles in root-hair development in Arabidopsis. Plant J **45:** 83–100

Kirik V, Simon M, Huelskamp M, Schiefelbein J (2004a) The ENHANCER OF TRY AND CPC1 gene acts redundantly with TRIPTYCHON and CAPRICE in trichome and root hair cell patterning in Arabidopsis. Dev Biol **268:** 506–513

Kirik V, Simon M, Wester K, Schiefelbein J, Hulskamp M (2004b) ENHANCER of TRY and CPC 2 (ETC2) reveals redundancy in the region-specific control of trichome development of Arabidopsis. Plant Mol Biol **55:** 389–398

Kurata T, Kawabata-Awai C, Sakuradani E, Shimizu S, Okada K, Wada T (2003) The YORE-YORE gene regulates multiple aspects of epidermal cell differentiation in Arabidopsis. Plant J **36:** 55–66

Lange BM, Wildung MR, Stauber EJ, Sanchez C, Pouchnik D, Croteau R (2000) Probing essential oil biosynthesis and secretion by functional evaluation of expressed sequence tags from mint glandular trichomes. Proc Natl Acad Sci USA **97:** 2934–2939

Larkin JC, Brown ML, Schiefelbein J (2003) How do cells know what they want to be when they grow up? Lessons from epidermal patterning in Arabidopsis. Annu Rev Plant Biol **54:** 403–430

Larkin JC, Walker JD, Bolognesi-Winfield AC, Gray JC, Walker AR (1999) Allele-specific interactions between ttg and gl1 during trichome development in Arabidopsis thaliana. Genetics **151:** 1591–1604

Leonhardt N, Kwak JM, Robert N, Waner D, Leonhardt G, Schroeder JI (2004) Microarray expression analyses of *Arabidopsis* guard cells and isolation of a recessive abscisic acid hypersensitive protein phosphatase 2C mutant. Plant Cell **16:** 596–615

Lloyd AM, Walbot V, Davis RW (1992) Arabidopsis and Nicotiana anthocyanin production activated by maize regulators R and C1. Science **258:** 1773–1775

Lukowitz W, Gillmor CS, Scheible WR (2000) Positional cloning in Arabidopsis: why it feels good to have a genome initiative working for you. Plant Physiol **123:** 795–805

Luo D, Oppenheimer DG (1999) Genetic control of trichome branch number in Arabidopsis: the roles of the FURCA loci. Development **126:** 5547–5557

Madson M, Dunand C, Li X, Verma R, Vanzin GF, Caplan J, Shoue DA, Carpita NC, Reiter WD (2003) The MUR3 gene of *Arabidopsis* encodes a xyloglucan galactosyltransferase that is evolutionarily related to animal exostosins. Plant Cell **15:** 1662–1670

Maere S, Heymans K, Kuiper M (2005) BiNGO: a Cytoscape plugin to assess overrepresentation of Gene Ontology categories in biological networks. Bioinformatics **21:** 3448–3449

Marks MD (1997) Molecular genetic analysis of trichome development in Arabidopsis. Annu Rev Plant Physiol Plant Mol Biol **48:** 137–163

Martienssen RA (1998) Functional genomics: probing plant gene function and expression with transposons. Proc Natl Acad Sci USA **95:** 2021–2026

Martin C, Glover BJ (2007) Functional aspects of cell patterning in aerial epidermis. Curr Opin Plant Biol **10:** 70–82

Morohashi K, Zhao M, Yang M, Read B, Lloyd A, Lamb R, Grotewold E (2007) Participation of the Arabidopsis bHLH factor GL3 in trichome initiation regulatory events. Plant Physiol **145:** 736–746

Nakamura M, Katsumata H, Abe M, Yabe N, Komeda Y, Yamamoto KT, Takahashi T (2006) Characterization of the class IV homeodomain-leucine zipper gene family in Arabidopsis. Plant Physiol **141:** 1363–1375

Nawy T, Lee JY, Colinas J, Wang JY, Thongrod SC, Malamy JE, Birnbaum K, Benfey PN (2005) Transcriptional profile of the *Arabidopsis* root quiescent center. Plant Cell **17:** 1908–1925

Noda K, Glover BJ, Linstead P, Martin C (1994) Flower colour intensity depends on specialized cell shape controlled by a Myb-related transcription factor. Nature **369:** 661–664

Payne CT, Zhang F, Lloyd AM (2000) GL3 encodes a bHLH protein that regulates trichome development in Arabidopsis through interaction with GL1 and TTG1. Genetics **156:** 1349–1362

Peres A, Churchman ML, Hariharan S, Himanen K, Verkest A, Vandepoele K, Magyar Z, Hatzfeld Y, Van Der Schueren E, Beemster GTS, et al (2007) Novel plant-specific cyclin-dependent kinase inhibitors induced by biotic and abiotic stresses. J Biol Chem **282:** 25588–25596

Pesch M, Hulskamp M (2004) Creating a two-dimensional pattern de novo during Arabidopsis trichome and root hair initiation. Curr Opin Genet Dev **14:** 422–427

Pina C, Pinto F, Feijo JA, Becker JD (2005) Gene family analysis of the Arabidopsis pollen transcriptome reveals biological implications for cell growth, division control, and gene expression regulation. Plant Physiol **138:** 744–756

Reiter WD, Chapple C, Somerville CR (1997) Mutants of Arabidopsis thaliana with altered cell wall polysaccharide composition. Plant J **12:** 335–345

Rosso MG, Li Y, Strizhov N, Reiss B, Dekker K, Weisshaar B (2003) An Arabidopsis thaliana T-DNA mutagenized population (GABI-Kat) for flanking sequence tag-based reverse genetics. Plant Mol Biol **53:** 247–259

Rumbolz J, Kassemeyer HH, Steinmetz V, Deising HB, Mendgen K, Mathys D, Wirtz S, Guggenheim R (1999) Differentiation of infection structures of the powdery mildew fungus Uncinula necator and adhesion to the host cuticle. Can J Bot **78:** 409–421

Saedler R, Zimmermann I, Mutondo M, Hulskamp M (2004) The Arabidopsis KLUNKER gene controls cell shape changes and encodes the AtSRA1 homolog. Plant Mol Biol **56:** 775–782

Schellmann S, Hulskamp M (2005) Epidermal differentiation: trichomes in Arabidopsis as a model system. Int J Dev Biol **49:** 579–584

Schellmann S, Hulskamp M, Uhrig J (2007) Epidermal pattern formation in the root and shoot of Arabidopsis. Biochem Soc Trans **35:** 146–148

Schellmann S, Schnittger A, Kirik V, Wada T, Okada K, Beermann A, Thumfahrt J, Jurgens G, Hulskamp M (2002) TRIPTYCHON and CAPRICE mediate lateral inhibition during trichome and root hair patterning in Arabidopsis. EMBO J **21:** 5036–5046

Schnittger A, Folkers U, Schwab B, Jurgens G, Hulskamp M (1999) Generation of a spacing pattern: the role of triptychon in trichome patterning in *Arabidopsis*. Plant Cell **11:** 1105–1116

Schnittger A, Weinl C, Bouyer D, Schobinger U, Hulskamp M (2003) Misexpression of the cyclin-dependent kinase inhibitor ICK1/KRP1 in single-celled *Arabidopsis* trichomes reduces endoreduplication and cell size and induces cell death. Plant Cell **15:** 303–315

Serna L, Martin C (2006) Trichomes: different regulatory networks lead to convergent structures. Trends Plant Sci **11:** 274–280

Simon M, Lee MM, Lin Y, Gish L, Schiefelbein J (2007) Distinct and overlapping roles of single-repeat MYB genes in root epidermal patterning. Dev Biol **311:** 566–578

Stracke R, Werber M, Weisshaar B (2001) The R2R3-MYB gene family in Arabidopsis thaliana. Curr Opin Plant Biol **4:** 447–456

Szymanski DB (2005) Breaking the WAVE complex: the point of Arabidopsis trichomes. Curr Opin Plant Biol **8:** 103–112

Szymanski DB, Jilk RA, Pollock SM, Marks MD (1998) Control of GL2 expression in Arabidopsis leaves and trichomes. Development **125:** 1161–1171

Szymanski DB, Lloyd AM, Marks MD (2000) Progress in the molecular genetic analysis of trichome initiation and morphogenesis in Arabidopsis. Trends Plant Sci **5:** 214–219

Tominaga R, Iwata M, Sano R, Inoue K, Okada K, Wada T (2008) Arabidopsis CAPRICE-LIKE MYB 3 (CPL3) controls endoreduplication and flowering development in addition to trichome and root hair formation. Development **135:** 1335–45

Uphof JC (1962) Plant Hairs. Gebrueder Borntraeger, Berlin

Vanzin GF, Madson M, Carpita NC, Raikhel NV, Keegstra K, Reiter WD (2002) The mur2 mutant of Arabidopsis thaliana lacks fucosylated xyloglucan because of a lesion in fucosyltransferase AtFUT1. Proc Natl Acad Sci USA **99:** 3340–3345

Wada T, Tachibana T, Shimura Y, Okada K (1997) Epidermal cell differentiation in Arabidopsis determined by a Myb homolog, CPC. Science **277:** 1113–1116

Wagner GJ, Wang E, Shepherd RW (2004) New approaches for studying and exploiting an old protuberance, the plant trichome. Ann Bot (Lond) **93:** 3–11

Walker JD, Oppenheimer DG, Concienne J, Larkin JC (2000) SIAMESE,

a gene controlling the endoreduplication cell cycle in Arabidopsis thaliana trichomes. Development **127:** 3931–3940

Wang S, Kwak SH, Zeng Q, Ellis BE, Chen XY, Schiefelbein J, Chen JG (2007) TRICHOMELESS1 regulates trichome patterning by suppressing GLABRA1 in Arabidopsis. Development **134:** 3873–3882

Wang S, Wang JW, Yu N, Li CH, Luo B, Gou JY, Wang LJ, Chen XY (2004) Control of plant trichome development by a cotton fiber MYB gene. Plant Cell **16:** 2323–2334

Weinl C, Marquardt S, Kuijt SJ, Nowack MK, Jakoby MJ, Hulskamp M, Schnittger A (2005) Novel functions of plant cyclin-dependent kinase inhibitors, ICK1/KRP1, can act non-cell-autonomously and inhibit entry into mitosis. Plant Cell **17:** 1704–1722

Wellesen K, Durst F, Pinot F, Benveniste I, Nettesheim K, Wisman E, Steiner-Lange S, Saedler H, Yephremov A (2001) Functional analysis of the LACERATA gene of Arabidopsis provides evidence for different roles of fatty acid omega-hydroxylation in development. Proc Natl Acad Sci USA **98:** 9694–9699

Wilkins TA, Rajasekaran K, Anderson DM (2000) Cotton biotechnology. Crit Rev Plant Sci **19:** 511–550

Wu A, Liu J (2006) Isolation of the promoter of a cotton beta-galactosidase gene (GhGal1) and its expression in transgenic tobacco plants. Sci China C Life Sci **49:** 105–114

Wu Y, Machado AC, White RG, Llewellyn DJ, Dennis ES (2006) Expression profiling identifies genes expressed early during lint fibre initiation in cotton. Plant Cell Physiol **47:** 107–127

Xia Y, Nikolau BJ, Schnable PS (1997) Developmental and hormonal regulation of the Arabidopsis CER2 gene that codes for a nuclear-localized protein required for the normal accumulation of cuticular waxes. Plant Physiol **115:** 925–937

Yephremov A, Wisman E, Huijser P, Huijser C, Wellesen K, Saedler H (1999) Characterization of the FIDDLEHEAD gene of *Arabidopsis* reveals a link between adhesion response and cell differentiation in the epidermis. Plant Cell **11:** 2187–2201

Zhang F, Gonzalez A, Zhao M, Payne CT, Lloyd A (2003) A network of redundant bHLH proteins functions in all TTG1-dependent pathways of Arabidopsis. Development **130:** 4859–4869

Zhang X, Oppenheimer DG (2004) A simple and efficient method for isolating trichomes for downstream analyses. Plant Cell Physiol **45:** 221–224

Zhao M, Morohashi K, Hatlestad G, Grotewold E, Lloyd A (2008) The TTG1-bHLH-MYB complex controls trichome cell fate and patterning through direct targeting of regulatory loci. Development **135:** 1991–1999

Zheng H, Rowland O, Kunst L (2005) Disruptions of the *Arabidopsis* Enoyl-CoA reductase gene reveal an essential role for very-long-chain fatty acid synthesis in cell expansion during plant morphogenesis. Plant Cell **17:** 1467–1481