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Research article

Gene expression profiling of the different stages of *Arabidopsis thaliana* trichome development on the single cell level☆

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Abstract

Leaf hairs (trichomes) of *Arabidopsis thaliana* are a model system for studying cell development, differentiation and cell cycle regulation. To exploit this model system with ultimate spatial resolution we applied single cell sampling, thus avoiding the averaging effect induced by complex tissue mixtures. In particular, we analysed gene expression profiles of two selected stages of the developing trichome: trichome initial cells and mature trichomes, as well as pavement cells. Ten single cells per sample were collected by glass microcapillaries and used for the generation of radioactive probes for subsequent hybridization to nylon filters representing approximately 8000 genes of *A. thaliana*. Functional categorization of genes transcribed in trichome initials, mature trichomes and pavement cells demonstrated involvement of these surface cells in the stress response. In silico promoter analysis of genes preferentially expressed in trichome initials revealed enrichment in MYB-binding sites and presence of elements involved in hormonal, metal, sulphur response and cell cycle regulation. Three candidate genes preferentially expressed in trichome initials were selected for further analysis: At3g16980 (putative RNA polymerase II), At5g15230 (*GASA4*) and At4g27260 (*GH3.5*, *WES1*). Promoter:*GUS* studies confirmed expression of the putative RNA polymerase II and the gibberellin responsive *GASA4* in trichome initials and partially in mature trichomes. Functional implication of the three selected candidates in trichome development and hence in cell cycle regulation in *A. thaliana* is discussed. We suggest that these genes are involved in differentiation and initiation of endocycling during trichome development.

Keywords: *Arabidopsis thaliana*; Single cell level; Gene expression profiling; Cell cycle; Plant hormones; Leaf trichomes; Trichome initial cells

1. Introduction

Trichomes are a suitable model system to study various aspects of cell differentiation including cell fate determination, cell cycle regulation, cell polarity induction and cell expansion growth [18,26,38]. Trichomes of *Arabidopsis* are single-celled structures of epidermal origin normally present on leaves, stems and sepals. Leaf trichomes are easily accessible and dispensable. Several regulatory genes involved in trichome patterning and development were identified by screening of trichome mutations. Some of these genes encode MYB-related transcription factors. These are positive regulators of trichome development such as *GLABRA 1* (*GL1*) and negative regulators as *TRIPTYCHON* (*TRY*), *CAPRICE* (*CPC*) and *ENHANCER TRIPTYCHON CAPRICE 1* (*ETC1*) (reviewed in [17]).

After being committed to the trichome pathway protodermal cells exit mitosis and start endoreduplication cycles reaching ploidy levels of 32C [18,19]. This process was described

Abbreviations: ABA, abscisic acid; GA, gibberellic acid; GFP, green fluorescent protein; GUS, beta-glucuronidase.

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as being regulated by the plant hormone gibberellic acid (GA), which was also postulated to be a key regulator of trichome initiation [31]. Further regulators of trichome endoreduplication are the *SIAMESE* (*SIM*) gene product [8,41], cyclins and inhibitors of cyclin-dependent kinases (ICK/KRP proteins). Expression of either *cycB1;2* or D-type cyclins under control of a trichome specific promoter caused emergence of multicellular trichomes [33,34]. Upon expression of inhibitors of cyclin-dependent kinases (ICK/KRP proteins) under control of a trichome specific promoter abortion of endoreduplication and subsequent cell death was observed [35]. Furthermore, endoreduplication is controlled by the trichome patterning genes, *GLABRA 3* (*GL3*) and *TRY* [14], protein degradation mechanisms [12,30] and DNA catenation mechanisms (DNA Topoisomerase VI).

In recent years, studies in developmental biology and "omics" approaches shifted towards investigation of single cells aiming to dissect more precisely processes involved in cell development, differentiation, cell function regulation, etc. Gene expression profiling data obtained from whole organisms, organs or even specific tissue types represent averaged signals due to their mixing of different cell types. Thus, these data ignore the fact that each organ of a multicellular organism is unique at the level of its tissues, cells and gene expression profiles. Furthermore, responses to environmental stimuli or developmental signals occur differentially at the single cell or tissue level. Investigation of the response of single cells to the plant hormone abscisic acid (ABA) provided substantial support for cell specific gene regulation [23]. This study demonstrated that only 20% and 27% of genes induced by ABA in mesophyll and guard cells, respectively, had been detected on the whole plant level. Similarly, 54% of the genes expressed in roots were found to be differentially expressed in root subzones [3], whereas only 37% of genes were differentially expressed among seven organs in maize [7]. These examples underline the urgent need for increasing spatial resolution in gene expression profiling, particularly down to the single cell level. However, single cell studies rely on efficient and precise methods for cell sampling and require special amplification technologies (reviewed by Brandt [6]). Microcapillary sampling has been successfully used for extraction of mRNA from single epidermal, mesophyll and companion cells [4]. For global transcriptional profiling, 20 mesophyll and 50 epidermal cells were sampled by microcapillaries and extracts were exploited for cDNA synthesis and microarray probe generation [5]. Moreover, this method was applied to study plant–pathogen interactions at the single cell level [27].

The aim of the present study was to discover novel pathways involved in trichome development and cell cycle regulation by identifying new candidate genes expressed in early stages of trichome development. High spatial resolution in transcript profiling was obtained by using microcapillary single cell sampling of pavement cells, trichome initials and mature trichomes. We hybridized cDNA probes of sampled single cells (10 cells per probe) to nylon filters containing 16,000 cDNAs from expressed sequence tags (ESTs) representing approximately 8000 genes of *Arabidopsis thaliana*. This enabled the identification of genes differentially expressed within two defined stages of trichome development. Using promoter:*GUS* studies and qRT-PCR we investigated the expression pattern of genes, overexpressed in trichome initials: At3g16980 (putative DNA-directed RNA polymerase II), gibberellin-regulated At5g15230 (*GASA4*) and auxin-responsive At4g27260 (*GH3.5*, *WES1*). Our results suggest involvement of the selected candidate genes in trichome development. In silico promoter analysis of the selected candidate genes propose novel interactions and common regulating mechanisms during trichome development.

2. Results

2.1. Workflow

After sampling of single trichome initials, mature trichomes and pavement cells we amplified the extracted mRNA populations, radioactively labelled it and proceeded with filter array hybridization. Latter was performed using material from three biological replicates. Candidate genes were selected based on ratios of differential gene expression within pairs of compared cell types. The expression pattern of our selected candidate genes was further investigated and confirmed by *real time* RT-PCR and histochemical GUS assays. To gain additional information on the possible interaction between our candidate genes and the cell cycle machinery we analysed the promoter regions of these genes in silico.

2.2. Single cell sampling and array hybridization

To label trichome initial cells we used transgenic *Arabidopsis* plants that expressed GFP (green fluorescent protein) under the control of the *GLABRA 2* (*GL2*) promoter (*pGL2*:*GFP*) (a gift from Prof. Martin Hulskamp, University of Cologne). *GL2* expression has been demonstrated in early stages of trichome development when there are still no visible signs of trichome formation ([37], Fig. 1). Furthermore, *GL2* is required for normal trichome morphogenesis. The GFP signal localized to the nucleus since the *pGL2*:*GFP* construct contained a nuclear localization sequence (NLS; Fig. 1).

GFP fluorescent labelling enabled the detection and sampling of incipient trichomes in the initial stage of their development. Samples of single trichome initial cells, mature trichomes and pavement cells contained a volume of 5 pl. Ten single cell extracts were merged, radioactively labelled and hybridized on array filters. In total nine filters were hybridized, three filters per cell type using three biological replicates.

Genes identified in extracts from trichome initial cells, mature trichomes and pavement cells were subsequently grouped into functional clusters (separately for each cell type) using Gene Ontology annotation available at the TAIR database (GO Biological Process). Comparison between pavement, trichome initial and mature trichome cells revealed only small differences in distribution of functional categories (Fig. 2).