Polarized Cell Expansion and Heterochronic shift in Angiosperm Spermatogenesis

A perspective from N. tabacum pollen transcriptome

Institute of Experimental Botany

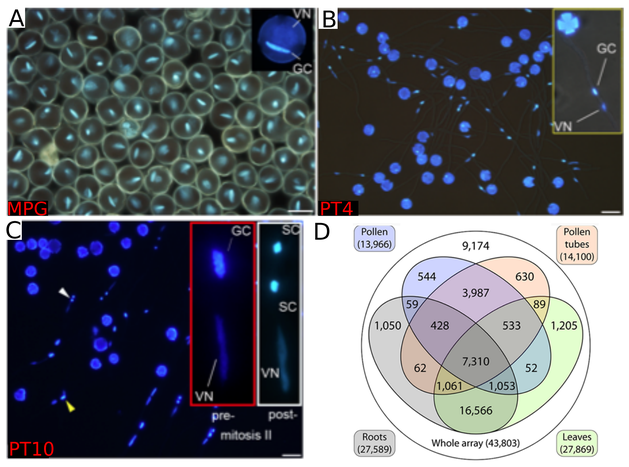
Said Hafidh[1], Katarina Breznenova[1], Jana Fecikova[1], Vera Capkova[1] and David Honys[1].

[1] Laboratory of Pollen Biology, Institute of Experimental Botany, Academy of Sciences, Rozvojova 263, 165 02 Prague, Czech Republic

1 Overview

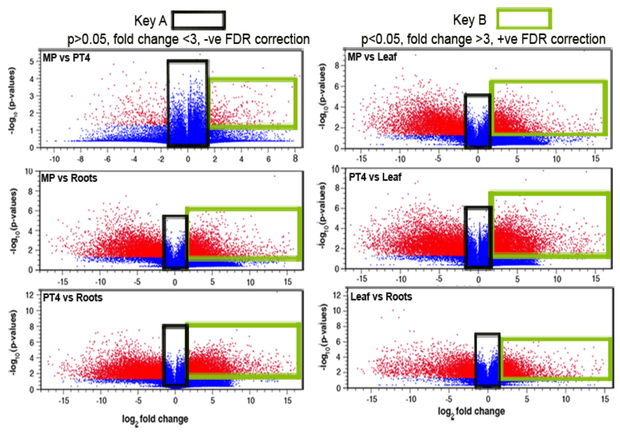
Single cell expansion of the growing pollen tube and the coordinated control of germ cell division and sperm cell fate specification, are landmark features of the developing male gametophyte. We applied the Agilent 44K tobacco gene chip to conduct the first transcriptomic analysis of the tobacco male gametophyte. We have performed a comparative study with the Arabidopsis root-hair trichoblast transcriptome to evaluate genetic factors and common pathways in polarized cell-tip expansion. Extension of our analysis beyond the second haploid mitosis allowed construction of a genetic model likely to have instigate the heterochronic shift in Angiosperm spermatogenesis.

2 Methods and material



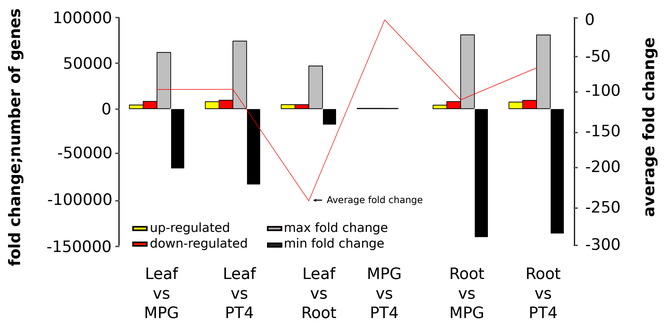
Microarray analysis was performed at two time points: mature pollen grains (MPG) and *in vitro*-germinated pollen tubes grown for 4 h (PT4). Pollen tubes grown between 4 to 48 h were used to establish dynamic expression of core cell cycle regulators. (D) Venn diagram of reliably detected probes.

3 Results 1: Divergent expression



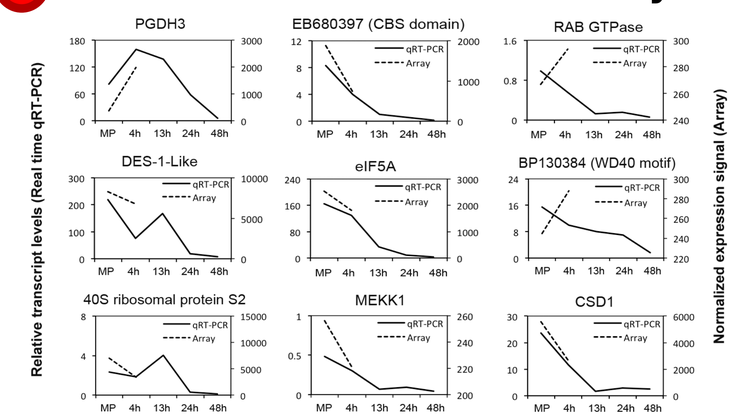
Gene enrichment analysis. We selected only genes fulfilling the following criteria: for a gene to have undergone a significant change in expression, a probe must have (1) a *p*-value ≤ 0.05; (2) passed a false discovery rate (FDR) correction to minimize the FDR; (3) an absolute expression signal (fold change) of ≥ 3 (up- or down-regulated); and (4) expression levels of >100 (arbitary cut-off).

4 Results 2: Summary of expression



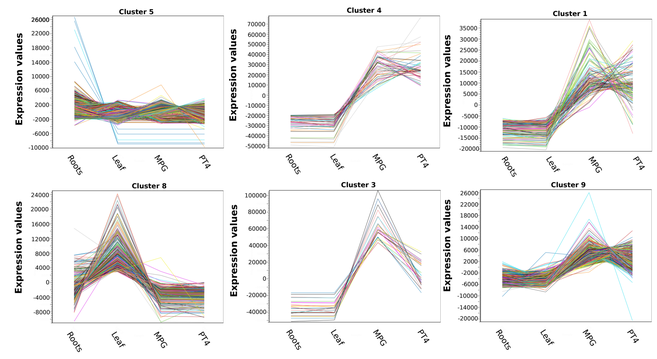
A moderate number of genes (830; 5.3% of male-gametophytic genes) had undergone a significant change in expression between MPG and PT4. Interestingly, the MPG transcriptome was 11.2% and 10.9% less diverse than the PT4 transcriptome when compared with leaves and roots, respectively.

5 Results 3: Validation of arrays



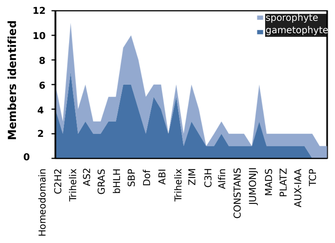
Pollen tubes grown for 4 to 48 h *in vitro* were used to verify the microarray expression by qRT-PCR as well as to extend their expression profile up to two days of pollen tube growth.

6 Results 4: Expression clusters



Clusters of co-expressed genes (K-means clustering) are insightful of co-regulated cellular processes (at the single cell resolution as well as developmentally) by a group of genes and thus useful information in building genetic maps of "functional interaction".

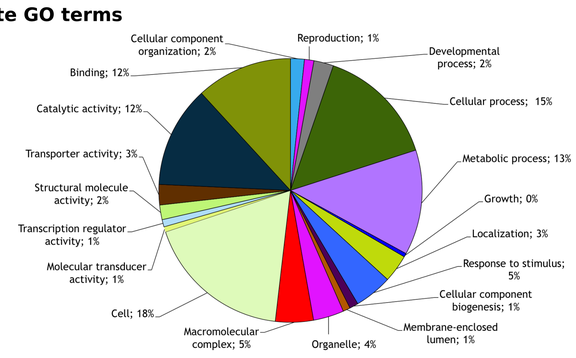
7 Results 5: TF families



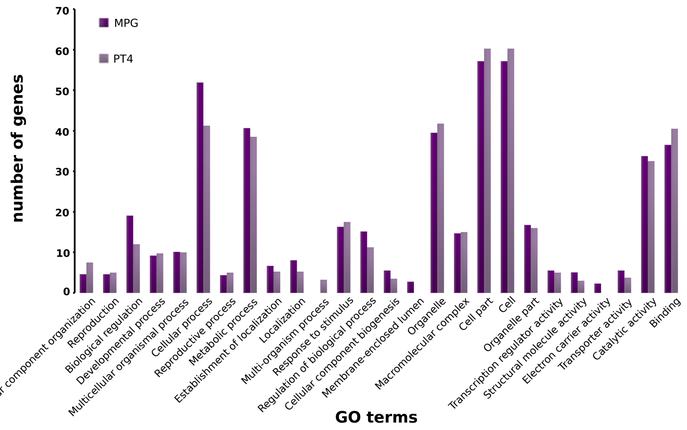
Diversity of some of the identified TF families in MPG and 4 h pollen tubes.

8 Results 6: GO terms distribution

Gametophyte GO terms

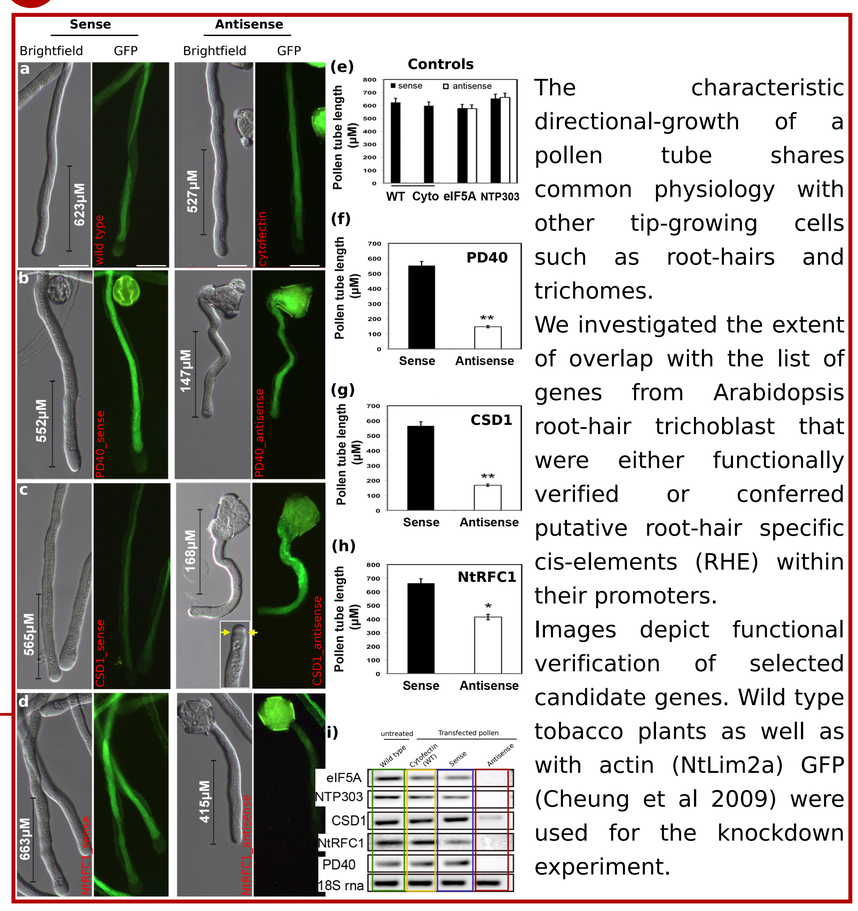


Dry pollen vs Pollen tube: overrepresented GO terms



Over-represented GO terms identified included those related to localization (GO biological process, p = 2.3e-11), transporter and structural molecule activities (GO molecular function, p = 1.2e-06 and p = 4.4e-16), and membrane-enclosed lumen (GO cellular component, p = 9.4e-06). The GO categories related to transporter activities$^{(p = 0.0016)}$, calmodulin binding$^{(p = 0.019)}$ and the macromolecular complex$^{(p = 5.4e-4)}$ were among the most overepresented in MPG compared with PT4.

9 Results 7: Polarized cell expansion

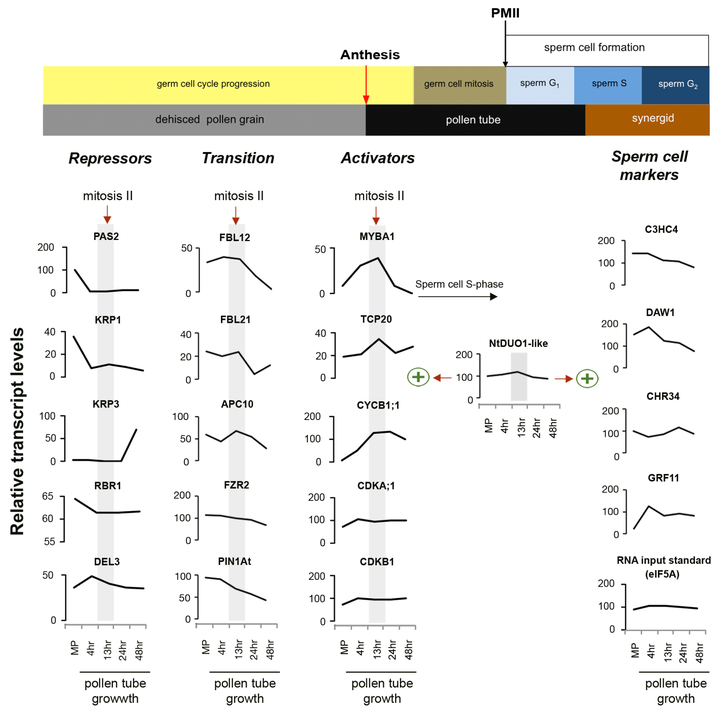


The characteristic directional-growth of a pollen tube shares common physiology with other tip-growing cells such as root-hairs and trichomes.

We investigated the extent of overlap with the list of genes from Arabidopsis root-hair trichoblast that were either functionally verified or conferred putative root-hair specific cis-elements (RHE) within their promoters.

Images depict functional verification of selected candidate genes. Wild type tobacco plants as well as with actin (NtLim2a) GFP (Cheung et al 2009) were used for the knockdown experiment.

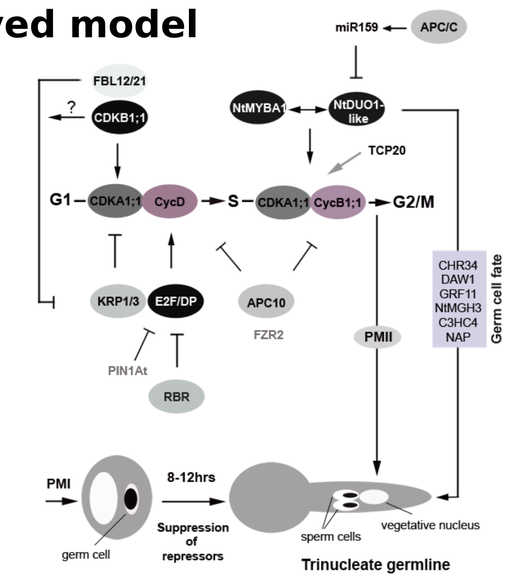
10 Results 8: Germ cell cycle modulators



We found genes with demonstrated or potential role in cell-cycle repression to be dominant in mature pollen and 4 h pollen tubes. Reduced accumulation of repressors >10 h after pollen tube growth implies de-repression and mitotic entry. A significant peak of G2/M factors (grey bars) marks transition to germ cell mitotic division.

In sync, sperm cell specification markers continuously accumulate pre- and post-division of the germ cell. A Myb TF (DUO1) has been shown in *Arabidopsis* to provide the link between germ cell division and fate specification.

11 Derived model



A proposed model reminiscent of a biological "clock" through which male and female gametes synchronizes cell-cycle progression for successful fertilization. This model provides a foundation to understand the molecular basis of the evolution of pollen tricellularity. The pathways acts as an informative 'genetic map' for experimental models to produce apomorphic tricellular pollen.

12 Perspectives

The expression dataset generated in this study provide an ideal platform for future research into aspects of polarized cell expansion and control of spermatogenesis. Cataloguing expressed genes will offer development of markers to study cell behaviour and cell fate. It also inspires development and improvement of current *in vitro* fertilization protocols that are of great interest to plant researchers, breeders and conservationists.

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Contact: hafidh@ueb.cas.cz