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Four recent papers have characterized the transcription profile of pollen grains, showing striking differences between gene expression in pollen and other plant tissues. These studies increase the number of known pollen-expressed genes by as much as 50-fold and have identified many novel genes that are potentially pollen-specific.

# Unveiling the gene-expression profile of pollen

José António da Costa-Nunes and Ueli Grossniklaus

Address: Institute for Plant Biology and Zürich-Basel Plant Science Centre, University of Zürich, Zollikerstrasse 107, CH-8008 Zürich, Switzerland.

Correspondence: Ueli Grossniklaus. E-mail: grossnik@botinst.unizh.ch

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## Abstract

Four recent papers have characterized the transcription profile of pollen grains, showing striking differences between gene expression in pollen and other plant tissues. These studies increase the number of known pollen-expressed genes by as much as 50-fold and have identified many novel genes that are potentially pollen-specific.

## Pollen development

The plant life cycle alternates between a diploid generation (the spore-producing sporophytes) and a haploid generation (the gamete-producing gametophytes). Unlike the situation in animals, in which the products of meiosis differentiate directly into gametes, the meiotic products of higher plants (called spores) undergo mitotic divisions to form multicellular haploid gametes [1]. The male gametophyte, pollen, is a highly specialized reproductive entity that performs a wide range of developmental functions, including cell specification and differentiation, cellular recognition, rapid polarized growth, chemotactic sensing, and fertilization [2].

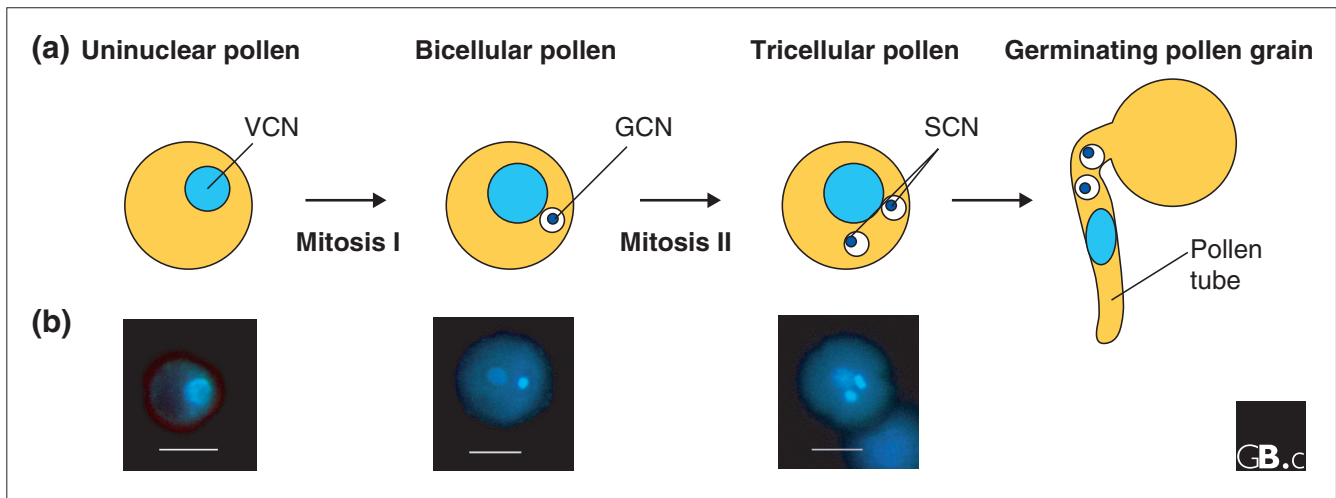
In flowering plants, the pollen grains are formed in the male reproductive organs of the flower, the anthers. The first mitotic division of the meiotic products (unicellular pollen) gives rise to two cells, the generative cell and the vegetative cell (Figure 1). The generative cell, which becomes engulfed by the vegetative cell, undergoes a second mitotic division, producing two sperm cells [3,4]. In some species, such as *Zea mays* (maize) and *Arabidopsis thaliana*, the second mitotic division of pollen occurs within the anther before release of pollen from the anthers (dehiscence) and pollen germination [4,5], whereas in others, such as *Nicotiana tabacum* (tobacco), this second mitosis does not occur until pollen tube germination [4]. After germination the pollen tube grows into female tissues. The pollen tubes grow

rapidly (up to 35 mm per hour), and are guided into the ovules, the precursors of seeds, where the two sperm are delivered to the two female reproductive cells, resulting in double fertilization [2,3].

The nucleus of the vegetative cell is larger than the nuclei of the sperm cells (Figure 1) [4,5]. This difference in size is generally attributed to a dramatic condensation of the chromatin in sperm cells, which has led to the belief that sperm are transcriptionally inactive [4]. They do not lack RNA, however; the characterization of this pool of RNA [6] may lead to the identification of factors that are essential for gamete fusion and/or the viability and the development of the zygote.

## Studying the expression profile of pollen

Until recently, very few pollen-expressed genes were known, and of these only 23 had been identified in *Arabidopsis* ([7] and references therein). Because the sequence of the *Arabidopsis* genome is complete, it is now possible to investigate the gene-expression profile of pollen on a more global scale for the first time [7-9]. Two different approaches were used to determine the overall gene-expression pattern of pollen: Affymetrix ATH1 8K GeneChips [7,8] and serial analysis of gene expression (SAGE) [9]. The fact that the ATH1 GeneChip [7,8] does not represent the entire *Arabidopsis*

**Figure 1**

Pollen-grain development. **(a)** A schematic representation of the stages of pollen development. **(b)** *Arabidopsis* pollen grains (Columbia accession), stained with DAPI to show DNA, at the stages indicated in (a). The larger uncondensed vegetative-cell nucleus (VCN; diffuse staining) and the condensed nuclei of one generative cell and two sperm cells (brighter dots in (b); GCN, generative-cell nucleus and SCN, sperm-cell nucleus) can be seen in the bicellular and tricellular pollen grains, respectively. The scale bar represents 10  $\mu$ m. Adapted from [4,5].

genome but only 8,200 genes - 30% of the recent estimate of 28,000 genes in the *Arabidopsis* genome [10] - suggests that more genes expressed in pollen remain to be identified. The SAGE approach [9] is expected to overcome this problem, however, as it can detect expressed RNAs from genes that are not represented on the GeneChip, including genes that are not even predicted or annotated (for comparisons of transcription profiling approaches see [11,12]). Surprisingly, perhaps, the two approaches [7-9] did give fairly similar overall views of pollen gene expression.

According to the data from the three reports [7-9], the pool of RNAs expressed in mature pollen is not just a haploid mimic of expression in the diploid sporophyte; in fact, quite the opposite. When expression data from seedlings, leaves, roots and/or siliques (seed pods) [8], plants at different developmental stages [7], and purified pollen grains [7,8] were compared, pollen turned out to have the most divergent expression profile of all [8]. This divergence is mainly due to the different levels of expression of individual genes [8], but also results from differences in expression between whole groups of genes with related functions [7]. These differences can partially be attributed to the fact that some genes are pollen-specific and others are expressed only in sporophytic tissues [7,8].

The most striking differences were the low expression levels in pollen of genes related to energy pathways (mainly photosynthesis) and translation [7,9]. This finding is not very surprising, as pollen is not photosynthetically active. The underrepresentation of transcripts involved in protein synthesis in mature *Arabidopsis* pollen grains is also in

accordance with previous biochemical and physiological experiments that suggest that pollen grains have a large pool of ribosomes and tRNA required for translation during rapid pollen-tube growth [3]. The other striking difference is the higher expression level of genes with proposed functions in signaling, cell-wall metabolism, and cytoskeletal dynamics in comparison with sporophytic tissues [7,9]. This enrichment fits with the requirement for interactions between the pollen grain and stigmatic cells prior to germination, rapid pollen-tube growth, and the attraction of the pollen tube towards the ovules that leads to double fertilization.

Another interesting aspect of studying global pollen expression is the identification of RNA molecules differentially present or expressed in the different developmental stages of the pollen grain [13,14]. These data allow investigation not only of which genes are expressed in pollen but also of the dynamics of their expression as pollen development proceeds.

### Pollen-specific gene expression

One interesting result of the recent reports [6-9] is the identification of pollen-specific genes, which may be involved directly in aspects of pollen biology, such as in pollen-tube growth and guidance or in fertilization. The numbers of genes specific to mature *Arabidopsis* pollen identified vary considerably between reports, partly perhaps because of differing plant growth conditions, pollen isolation and sorting protocols, ecotypes used, experimental set-up and data analysis, and criteria chosen to define the genes that are pollen-specific. The GeneChip papers [7,8] report that 10% or 40% of the genes expressed in *Arabidopsis* pollen are

pollen-specific (162 or 387 genes in [8] and [7], respectively); the SAGE paper [9] states that 83% of the pollen-expressed gene tags (1,251 tags) are pollen-specific (for a list of the genes found, see the online supplementary data of the three publications [7-9]). The three reports give estimates of the total number of genes expressed in mature *Arabidopsis* pollen that are just as different, ranging from 992 [7] to 1,587 [8]. Some of the pollen-specific genes not only are unique to pollen but also are among the genes that are most highly expressed in pollen [7,8], so they are the most likely to have a crucial function in pollen biology and are worth further investigation. More interestingly, the existence of several novel pollen-specific genes of unknown function was revealed [7-9], some of which were neither represented in expressed sequence tag libraries nor even annotated in the *Arabidopsis* genome [9], paving the way for new research.

As the Affymetrix ATH1 8K GeneChip includes only about 8,000 genes, it is likely that there are more pollen-expressed genes to be discovered. This indeed appears to be the case, as data obtained from the Affymetrix ATH1 24K GeneChip (which represents approximately 24,000 *Arabidopsis* genes) give 4.5 times as many pollen-specific and 4 times as many pollen-expressed genes as were found using the Affymetrix ATH1 8K GeneChip ([1] and C. Pina, J.A. Feijó and J.D. Becker, personal communication).

### Sperm-cell-specific gene expression

A fourth interesting recent study [6] used a cDNA library to identify sperm-specific transcripts, which may be involved in gamete-gamete recognition or early events after fertilization. Given that sperm cells have little cytoplasm and are believed to be transcriptionally inactive, and that the larger vegetative cell contains much more cytoplasm and an uncondensed nucleus expressing the 'late' pollen genes [3,6], the sperm RNA pool is expected to be underrepresented in pollen-grain cDNAs. In order to identify sperm-cell-specific RNAs, therefore, a library had to be made from isolated and purified sperm cells [6,15]. Analysis of this library (still in progress) has identified several transcripts that are present in both the vegetative and the sperm cells of maize [6]. A most interesting finding was the identification of transcripts from the mature pollen that accumulate only in sperm cells but not in the vegetative cell. *In situ* hybridization and reverse-transcriptase-coupled PCR data have shown that one such sperm-specific transcript (Zmsp041, a MtN3-like cDNA), despite being expressed in unicellular and bicellular pollen, is present only in the sperm cells in mature pollen. Despite its initially broad expression pattern, its presence exclusively in the sperm cell might indicate a role in fertilization [6].

The four recent reports [6-9] comprise an immense contribution to our current knowledge of pollen gene expression. They have revealed previously unknown pollen-specific transcripts that may be important for pollen biology. These and

future gene-expression studies of mature pollen, and in particular of sperm and vegetative cells, will contribute to the identification of genes required for rapid pollen tube growth and other aspects of pollen biology such as pollen-stigma interaction, pollen-tube guidance and double fertilization. This new wealth of pollen transcription data (an area in which *Arabidopsis* research was lagging behind [8]) can now be used for functional studies. The data also lay the foundation for studying changes in expression under different conditions, for example cold stress and drought [9], and for bioinformatic analyses of the regulation of pollen-expressed genes [16], which could, in the long term, have practical agricultural applications.

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