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Cell-specific expression profiling of rare cell types as exemplified by its impact on our understanding of female gametophyte development

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Expression profiling of single cells can yield insights into cell specification, cellular differentiation processes, and cell type-specific responses to environmental stimuli. Recent work has established excellent tools to perform genome-wide expression studies of individual cell types, even if the cells of interest occur at low frequency within an organ. We review the advances and impact of gene expression studies of rare cell types, as exemplified by recently gained insights into the development and function of the angiosperm female gametophyte. The detailed transcriptional characterization of different stages during female gametophyte development has significantly helped to improve our understanding of cellular specification or cell–cell communication processes. Next-generation sequencing approaches — used increasingly for expression profiling — will now allow for comparative approaches that focus on agriculturally, ecologically or evolutionarily relevant aspects of plant reproduction.

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Introduction

Cell specification, cellular differentiation, and specific cellular responses to environmental stimuli involve changes in gene expression. Therefore, a view of the transcriptome of a cell provides a snapshot of the cellular instruction machinery that strongly depends on developmental stage and environmental inputs. Recent technological developments have enabled genome-wide expression experiments at reasonable costs [1]. In addition, cell type-specific transcriptional profiling has dramatically improved our understanding of biological processes (e.g. reviewed in [2]). Two major lessons have been learnt from the analysis of genome-wide expression data in individual cell types or specific tissues in plants.

First, the cellular context is important when studying developmental processes, because cell-specific gene expression is generally masked when performing studies at the organ level (reviewed in [3]). Ground-breaking novel insights into the development of plants have been made by profiling essentially all cell types that occur in the *Arabidopsis* root [4–7], through studying male gametophyte development (pollen) [8], or by expression profiling during *Arabidopsis* female gametophyte (embryo sac) development [9,10•,11••,12••] (see also below).

Second, multiple lines of evidence suggest that not only cellular differentiation processes are best understood at the single-cell level, but also responses of an organism to environmental stimuli: in genome-wide expression studies strong interactions between cellular identity and environmental variation have been observed, for example when examining the effects of stress or nutrient treatments on different cell types in the root [13–15].

Here, we summarize the tools that are available to isolate specific cell types from heterogeneous tissues as well as advances in transcriptional profiling methods. Additional reviews on related topics have been published recently [2,3,16,17], but here we briefly summarize and compare the tools with a focus on their suitability for cells that occur at low frequencies within a tissue. We also discuss what insights have been gained through cell-specific gene expression profiling of rare cell types in plants, as exemplified by studies on female reproductive processes.

Techniques used for the isolation of individual cell types

Initial approaches for genome-wide expression profiling largely focused on the profiling technologies themselves, meaning that experiments were often performed at the whole-plant, organ, or tissue level [18]. Only for certain cell types, such as those of the male gametophyte, could specific stages be collected and profiled relatively easily [8]. In recent years however, the scientific community has been creative in generating a variety of methods to isolate and profile distinct cell types at specific developmental stages. Here, we summarize recently applied approaches — and identify limits, strengths and weaknesses of these — with a special focus on their

Table 1

Summary of popular cell isolation methods developed in recent years

Cell isolation method	Limits of relative cell occurrence	Relative enrichment scores of cells[b]	Technique/costs	Further applications	Use in non-model organisms?
Biochemical isolation	N/A [20,21]	N/A	Cheap	Many	Yes, but limited to few, selected cell types
FACS	~1% (down to 0.1% if highly optimized)	~9–60× [44••,89]	Expensive equipment, extensive protocol	Proteomics, metabolomics, genome-wide chromatin structure	Relies on transgenic lines
LAM	<0.1%	Depends on morphology (up to ~10,000×) [9]	Expensive equipment, long protocol	For rare cells: mostly limited to expression profiling (low throughput) but has also been used for DNA methylation analyses	Yes
INTACT/INTACT-derived	<1–10%[a]	100–170× [44••]/up to 10,000× [47••]	Easy protocol, cheap	Genome-wide chromatin structure	Relies on transgenic lines
Micromanipulation	<0.1%	Depends on accessibility of cells (~up to 10,000×) [48]	Technically challenging, depends on morphology of cell type	For rare cells: limited to expression profiling (due to low throughput)	Yes

[a] The limits of the method have not been tested thoroughly.
[b] Relative enrichment scores are defined here as [no. of target cells in output/no. of non-target cells in output]/[no. target cells in input/no. of non-target cells in input], e.g. from 10% relative fraction in input to 99% relative fraction in output: enrichment score = (99/1)/(10/90) = 891.

application to the collection of rare cell types (Table 1). For the use of methods in applications using frequent cell types, we refer to previous recent reviews [2,3,19].

Biochemical purification of selected cell types

It is possible to isolate certain cell types using mechanical and/or biochemical enrichment procedures. This approach is only applicable to selected cell types, for example, guard cells [20], trichomes [21] and sperm cells (MA Schauer, Protein dynamics of pollen development, PhD thesis, University of Zürich, 2010), with specifically designed methods for each. Since it is possible to isolate relatively large numbers of cells using such procedures, they are not only suitable for transcriptomics [20,21], but also for proteomics [22–24, MA Schauer, Protein dynamics of pollen development, PhD thesis, University of Zürich, 2010], metabolomics [25], analyses of DNA methylation [26], or the determination of cell wall composition [21].

Genetic subtraction methods

Hereby, a genetic background that alters developmental processes or cell type abundance in a tissue is employed, for instance mutants with an increased number of stomata [27], mutants with altered floral organs [28], floral mutants in combination with inducible transgenic constructs [29,30], or mutants missing the female gametophyte [31–34]. These tools are fairly limited to the biological system under study and often rely on specific mutants and/or transgenic backgrounds that may be difficult and time-consuming to establish. Furthermore, the approach is subtractive and, by definition, genes that are expressed both in the surrounding tissue and the target cells cannot be detected.

Fluorescence-activated sorting of cells or nuclei

The approach relies on automatically sorting cells [4,7,35] or nuclei [36] that are tagged with a fluorescent marker (such as the green fluorescent protein) using a flow cytometry system. The method results in high yields and good enrichments, and is suitable for transcriptomics [4,7,35,37], proteomics [38], and metabolomics [3]. However, the method is usually limited to cells that have a relative occurrence of more than 0.5% (0.1% under highly optimized conditions) within the harvested tissue (*Kenneth Birnbaum*, *personal communication*). This requirement impedes, for example, the isolation of female gametophytic cells from carpels or even from isolated ovules.

Cell-specific tagging of RNA, RNA-binding proteins, or components of the ribosome

In several experiments, specific tagging and pull-down of either RNA (e.g. in *Drosophila* [39]), RNA-binding proteins, or ribosomal components [40–43] has yielded insights into cell-specific processes. For example, in a study of cell-specific responses to environmental variation, it was shown how hypoxic stress affects the translatome (i.e. the mRNA population associated with the ribosome in the process of translation) [43]. However, these methods have so far been used for more frequently occurring cell types only, and their use for rare cell types may not feasible [39].