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Technologies for systems-level analysis of individual cell types in plants

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Abstract

The study of biological processes at cell type resolution requires the isolation of the specific cell types from an organism, but this presents a great technical challenge. In recent years a number of methods have been developed that allow deep analyses of the epigenome, transcriptome, and ribosome-associated mRNA populations in individual cell types. The application of these methods has lead to a clearer understanding of important issues in plant biology, including cell fate specification and cell type-specific responses to the environment. In this review, we discuss current mechanical- and affinity-based technologies available for isolation and analysis of individual cell types in a plant. The integration of these methods is proposed as a means of achieving a holistic view of cellular processes at all levels, from chromatin dynamics to metabolomics. Finally, we explore the limitations of current methods and the needs for future technological development.

Keywords

cell type; plant; transcriptome; epigenome; proteome; metabolome; profiling

1. Introduction

Plant organs are intricate structures composed of many physiologically and morphologically distinct types of cells. Each cell type has a particular function that is directed by its own unique transcriptome, proteome and metabolome. One of the crucial features of multicellular development is the establishment of a cell type-specific epigenome and transcriptome as cells differentiate and acquire particular fates [1]. In addition to normal development, it is now clear that individual cell types respond in different ways to environmental stresses and interactions with other organisms [2, 3].

Despite the unique behavior of each cell type in the plant, it has historically been a great technical challenge to analyze specific cell types in a comprehensive way. As a result, most

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studies on RNA, chromatin, proteins, or metabolites have analyzed whole tissues in an attempt to understand developmental processes or responses to the environment. While this approach has been extremely fruitful, it results in the amalgamation of signals from multiple cell types, and these cannot be deconvoluted to assess the behavior of individual cell types within the tissue. In animal model systems, this problem has been partly circumvented by using cultured cell lines of defined types, as well as triggering stem cells to differentiate into various specialized cell types in culture [4, 5]. In plants, there are limited examples of culturing and differentiation of specific cell types *ex vivo* [6]. Furthermore, such approaches take cells out of their native environment and are therefore likely to introduce artifactual changes in the biology of the cultured cells. In order to achieve a complete and reliable molecular description of the development and physiology of multicellular organisms, we must ultimately employ methods that allow the behavior of individual cell types to be examined as they are in their native environment within the body.

In this review, we describe currently available techniques that enable cell-type specific profiling in plants. Several recent reviews have extensively discussed the biological insights that have been gleaned through the application of cell type-specific profiling technologies [7-9], thus we focus here on the technical aspects of these methodologies. We discuss the parameters that can be measured using each technique, as well as how these methods could be integrated to provide a holistic description of cellular processes. We further discuss the limitations of current methods and the technical challenges that need to be addressed in the years ahead.

2. Mechanical methods for isolating specific cell types of an organism

The initial obstacle to cell type-specific profiling was the technical difficulty associated with purifying the desired cell types away from all others in the plant. Historically, two main strategies have been used to isolate specific cell types: dissection to obtain the desired cell type or disruption of a whole tissue followed by isolation of the cell type of interest based on unique physical properties. A variety of dissection methods have been employed, including simple peeling to isolate the leaf epidermis [10], the “Tape-Arabidopsis Sandwich” method which uses tape to separate leaves into different cell layers [11], or the “single-cell aspiration” method which uses microcapillary aspiration to isolate the cytoplasmic contents from a single cell [12-15] .

Additionally, studies on specific cell types have employed various strategies that disintegrate tissues into individual cells, by either enzymatic digestion or mechanical grinding, followed by the selection of certain cell populations based on properties such as their susceptibility to digestion, their size as selected through filtering, or by differential fluorescent labeling [16-18]. A classical example of such a strategy is the selective release of mesophyll cells from Asparagus cladophylls by mechanical grinding [19].

More modern methods that capitalize on the concepts of dissection or sorting are laser microdissection (LM) and fluorescence-activated cell sorting (FACS), which are based on mechanical and optical/mechanical separation mechanisms, respectively [20, 21]. These

methods have been used extensively in both animal and plant studies, with their main utility at this point being transcriptome profiling.

2.1 Laser microdissection (LM)

Laser microdissection (LM) is a direct tool for isolating specific cell types from sectioned tissues. In this method, a tissue is chemically fixed, embedded in a solid material, and sliced into thin sections to give access to the desired cell types. The cell type of interest can then be excised from the section and captured through the use of a laser microdissection apparatus. Under microscopic examination, a region containing the desired cells is defined within the software environment, and the instrument then uses a focused laser to cut around the border of the defined area and thereby separates it from the surrounding cells. This region containing the cells of interest can then be isolated by several different methods, including direct attachment to an adhesive surface or by catapulting the tissue out of the section and into a receptacle using a laser pulse (Fig. 1A) [22].

In most cases, LM has been used to analyze the RNA content of individual plant cell types in varied contexts, leading to important advances in our understanding of the transcriptomes that define specific cell populations. For example, recent studies have analyzed the transcriptomes of meristematic stem cells and organ primordia in maize [23], those of meristem cells before and after the floral transition in Arabidopsis [24], as well as 40 different rice cell types [25].

The LM technique has also been used to study plant-pathogen interactions including the life cycle of foliar rust fungus during infection of poplar leaves [26] and the response of *Medicago truncatula* roots to colonization by a mycorrhizal symbiont [3]. Excitingly, this latter study measured not only transcriptome changes that occur during colonization, but also proteomic changes as examined by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Collectively, this and several other studies describing MS-based proteomic analysis of microdissected plant cells [27] and tumor cells [28, 29] indicates that LM will allow both the transcriptome and proteome to be characterized at the resolution of individual cell types. Furthermore, gas chromatography-MS was used to identify dozens of metabolites in microdissected vascular bundles in Arabidopsis [30].

In terms of practice, LM has the advantage of being theoretically straightforward in that the desired cell type is purified directly from its native environment within the tissue, without requiring any knowledge of the molecular properties of the cell type. This is in stark contrast to the other methods described below, all of which require some prior knowledge about the cell type being studied. However, LM does have several significant drawbacks. These include the requirement for visual definition of specific cell types (which may be subjective and inaccurate) and the use of expensive instrumentation. Finally, LM provides an inherently low yield of cells, which is likely to be problematic for the measurement of certain parameters such as the epigenome and metabolome. However, with sensitive RNA amplification methods, LM-isolated material is highly amenable to transcriptome profiling.

2.2 Fluorescence-activated cell sorting (FACS)

Fluorescence-activated cell sorting is based on the optical identification and mechanical capture of fluorescently labeled cells. In this procedure, cells are completely dissociated from one another by protoplasting, and the cell type of interest is fluorescently labeled. This labeling can be achieved by the expression of a fluorescent protein from a cell type-specific promoter, by treatment of the cell mixture with a fluorescent antibody against a cell type-specific surface protein, or treatment with agents that fluoresce under certain cellular conditions [18]. This suspension of individual cells is forced through a small nozzle to produce a stream of droplets, each containing a single cell, and these droplets pass by a laser that is tuned to the excitation wavelength of the fluorescent molecule being used. If the droplet is fluorescent, and therefore contains a cell of the targeted type, a charge is applied to it so that it is forced out of the droplet stream and into a collection receptacle as it passes by charged deflecting plates [31] (Fig. 1B).

The general strategy for FACS isolation of specific plant cell types is to prepare protoplast suspensions for sorting by treating the tissue with a mixture of cell wall-degrading enzymes to remove the cell wall and then sort out the protoplasts of interest based on their expression of GFP from a cell type-specific promoter [32]. An alternative FACS-based approach is to sort a crude tissue homogenate in which nuclei from the desired cell type express a fluorescently-tagged histone protein [33]. The application of FACS to the study of specific plant cell types has now been used for transcriptome profiling of nearly all cell types in the Arabidopsis root [34, 35], as well as multiple domains within the apical meristem and floral organ primordia [36]. Several studies have also used FACS to analyze cell type-specific transcriptional responses to abiotic factors. Measurements made to date include the transcriptional responses of different Arabidopsis root cell types to deficiencies of iron [37] or sulfur [38], nitrogen fluctuations [2], as well as exposure to low pH [38] or high NaCl concentrations [37].

Beyond transcriptomics, another application for FACS has been in the measurement of the hormone auxin at cell type resolution in roots through MS analysis of sorted protoplasts [39]. This study suggests that FACS may be a generally useful tool for analyzing metabolites in individual cell types. More recently, proteomic measurements have also been made using FACS-purified root cell types [40].

Compared to LM, FACS can provide much higher cell yields. This simplifies its application to system-level profiling, particularly with regard to molecules other than nucleic acids, where no amplification technologies exist. However, with the increasing sensitivity of downstream analytical methods, yield may not be a crucial factor in the near future. Rather, purity and maintenance of cells in their original physiological condition may be more important factors to consider. LM and FACS share the common drawbacks of requiring time-consuming procedures and the use of expensive instrumentation. Furthermore, the use of FACS is complicated by autofluorescence from plant cell components, making it most amenable for use with non-autofluorescent cells such as those of the root. The use of fluorescent proteins that have no spectral overlap with plant autofluorescence, or sorting of nuclei, should allow FACS to be used on a wider range of tissues. However, a wealth of studies using FACS for root cell analyses and an underrepresentation of its use in other cell

types suggest that it is currently not ideal for most shoot cell types. Like the methods described below, FACS also requires prior knowledge of a promoter or enhancer trap that is specifically expressed in the desired cell type.

3. Affinity-based methods for analyzing the epigenome, transcriptome, and translatome of individual cell types

In contrast to the mechanical cell purification methods described above, several molecular affinity-based methods have been devised to purify subcellular components such as nuclei or ribosomes from individual cell types. The general advantage of these methods is that very little tissue manipulation is required, and they provide a fast system in which many purifications can be performed in parallel. These advantages come with the limitation that only particular organelles or subcellular structures can be isolated.

3.1 Isolation of nuclei tagged in specific cell types (INTACT)

The strategy behind the INTACT method is to place an affinity tag on the nuclear envelope in a desired cell type so that nuclei from that cell type can be specifically purified. The main component of this system is the nuclear tagging fusion protein, which consists of a nuclear envelope-associating domain, GFP, and the biotin ligase recognition peptide, which can be biotinylated by the *E. coli* biotin ligase, BirA. Expression of the tagging protein and BirA in the same cell therefore generates nuclei that are coated with a fluorescent, biotinylated protein. Those nuclei can then be affinity purified from a tissue homogenate using magnetic beads coated with streptavidin, a biotin-binding protein. This makes for a simple and robust purification that is performed by grinding tissue to isolate nuclei, adding magnetic streptavidin beads, and capturing bead-bound nuclei with a magnet (Fig. 1C) [41].

The INTACT method was first used to isolate nuclei specifically from the hair and non-hair cell types of the Arabidopsis root epidermis [38]. The nuclear tagging protein was expressed using a promoter specific to one of these cell types, along with constitutive expression of BirA. This system was used to analyze the genome-wide distribution of two different histone modifications as well as the nuclear transcriptome of each cell type [42]. RNA isolated from mammalian nuclei and Arabidopsis shoot nuclei was previously shown to have a very similar composition to that of total cellular RNA from each respective tissue [43, 44], and this was also found to be true of Arabidopsis roots [42]. Thus, while the INTACT method is ideal for epigenomic analyses, it also appears to give a representative picture of the total transcriptome of hair and non-hair cell types.

Compared to FACS and LM, INTACT has the advantages of being very rapid, requiring only standard laboratory equipment, and providing a high yield and purity of target nuclei. The INTACT method does have several significant drawbacks in that it requires a cell type-specific promoter and can only purify nuclei, which precludes the analysis of cytoplasmic contents. However, this approach may be useful for analyzing other nuclear features, such as the nuclear proteome. In addition, a similar strategy has been used to purify chloroplasts from specific cell types [45] and might be extended to other cellular organelles or even specific types of membranes.

3.2 Translating ribosome affinity purification (TRAP)

In the flow of information beyond the epigenome and transcriptome, mechanisms of post-transcriptional regulation can be studied by analyzing the mRNAs that associate with ribosomes. This has recently become possible at the level of individual cell types with the advent of TRAP. This method is based on expression of an epitope-tagged ribosomal protein from a cell type-specific promoter, which allows the selective purification of ribosomes from that cell type. Starting from a total ribosome preparation, labeled ribosomes from the cell type of interest are affinity purified on beads coated with an antibody specific to the epitope used to tag the ribosomal subunit [46] (Fig. 1D). The ribosome-associated mRNAs (collectively referred to as the translatome) that are recovered can then be analyzed by microarray or, preferably, deep sequencing. The TRAP method has already been used to study the translatomes of 21 different Arabidopsis cell types under normal and hypoxic conditions [47] as well as multiple zones of the Arabidopsis floral meristem and developing flowers [48].

The affinity-based purification methods, INTACT and TRAP, share the benefits of requiring very little manipulation of tissue prior to purification and providing relatively high yields of purified material. However, both of these methods do require prior knowledge of cell type-specific promoters and deliver only a subset of the cellular contents.

4. Integration of methods that measure different parameters: toward a holistic understanding of gene regulation, cell physiology, and metabolism

Each of the cell type-specific profiling methods described above is particularly well suited to measure one or more cellular parameters (Fig. 2), and most studies to date have measured a single parameter in a given cell type. However, to fully understand the inner workings of a given cell type, it will be necessary to describe its genome, epigenome, transcriptome, proteome, and metabolome in a quantitative manner. This should be possible by taking advantage of the strengths of multiple cell type-specific profiling methods to examine all of these parameters in any given cell type.

4.1 The nuclear genome

A commonly accepted but largely untested notion is that every cell in a multicellular organism has exactly the same genome sequence. However, there are multiple lines of evidence that suggest that this may not always be the case. For example, it is well established that endoreduplication leads to different ploidy levels in plant cells [49], but whether the entire genome is always fully replicated during this process is not known. In the case of Drosophila polytene chromosomes, centromeres and heterochromatic regions do not replicate to the same extent as other genomic regions, leading to imbalances in genome representation [50]. If such a phenomenon occurs during plant cell endoreduplication, it could lead to variations in gene dosage and perhaps have significant biological effects. Furthermore, there is evidence for transposon reactivation specifically in pollen vegetative nuclei [51], which might also occur in other plant cell types, leading to gene disruptions and/or genomic rearrangements. While all plant cells appear to be totipotent, suggesting

exceptional genome stability, genomic variations between cells could underlie the phenomenon of somaclonal variation in plants regenerated from cultured cells [52].

It will be interesting to determine whether the genome differs between distinct cell populations within the same plant and how this could affect cellular activities. The techniques of FACS, INTACT, and perhaps LM, would be well suited for sequencing the genomes of individual cell types.

4.2 The epigenome and transcription kinetics

Gene regulatory networks, including transcription factors and chromatin components such as histone post-translational modifications, histone variants, and nucleosome remodeling enzymes, are responsible for controlling the transcriptional output of the genome [53]. Yet how these factors work together in a given cell type to establish, maintain, or switch the on/off state of its genes is still not well understood. We now have the tools to examine the actions and interactions of these factors at cell type resolution. This will provide a deeper understanding of how cell type-specific transcriptional programs are established and how individual cell populations respond to the environment at the level of gene transcription.

The INTACT method is particularly well suited for analyses of the epigenome, given that it can be used to specifically purify nuclei from a desired cell type. The isolated nuclei could be used to comprehensively map the genomic location of transcription factors, RNA polymerase, histone post-translational modifications, histone variants, nucleosomes, as well as nuclease hypersensitive sites through next-generation sequencing. In addition, INTACT might be used in conjunction with the recently developed CATCH-IT method for measuring the kinetics of nucleosome disruption and replacement [54], allowing this process to be studied at cell type resolution.

It will also be useful to measure transcription kinetics and dynamic RNA polymerase pausing across the genome to understand the connections between the epigenome and transcription. This might be possible by combining INTACT with a recently developed method for genome wide run-on sequencing (GRO-seq) [55]. It should also be possible to use FACS-purified cells in these types of epigenomic assays.

4.3 The steady-state transcriptome and translatome

The steady-state transcriptome and the association of mRNAs with ribosomes lie on the spectrum of gene expression beyond the epigenome and nascent transcriptome. Ultimately, it will be necessary to measure the steady-state transcriptome and translatome of each cell type to understand what makes a given cell type unique and to uncover the mechanisms of post-transcriptional regulation at the levels of splicing and ribosome association.

The steady-state transcriptome can be comprehensively measured by sequencing RNA from cells isolated by LM or FACS, or nuclei isolated by INTACT. FACS and LM may be better suited to make such measurements given that they are able to capture the total cellular RNA pool. This will be particularly true with respect to small RNAs that are not found in the nucleus. Conversely, the use of INTACT to isolate nuclear RNA may be desirable for the study of nuclear small RNA pathways [56].

The translatome can be characterized and quantified through the use of TRAP in conjunction with RNA-seq. Together these measurements will provide a comprehensive view of the steady-state levels of RNAs in various cell types, including unique splice variants or alternative transcripts from a gene as well as the profile of mRNAs that are undergoing translation. Collectively, this information will define the transcriptome of each cell type and allow the generation of hypotheses regarding post-transcriptional regulatory mechanisms that are at play in that cell type.

4.4 Proteomics and metabolomics

In the sense that DNA itself simply encodes the toolkit for building a cell and carrying out its function, the proteome is the ultimate representation of cell identity, and the metabolome follows from it. It is at these levels that we can understand the physiology, metabolism, and function of each cell type in the body. Unfortunately, the proteome and metabolome are much more difficult to measure compared to nucleic acids, mainly due to problems with measurement sensitivity.

Unlike the analysis of nucleic acids, there are no amplification methods available to increase the quantity of proteins or metabolites prior to analysis. At present, a small fraction of the proteome and metabolome can be identified and quantitatively measured [9]. The tools to do so at the resolution of individual cell types are available, but have not been widely used at this level. Improvements in instrument sensitivity as well as increases in the depth of plant-specific MS libraries are helping to solve this problem [57].

In plants, cell type-specific proteome measurements have been made using LM-isolated material [3], and this could be a generally useful strategy as it has also been used to analyze protein content in very small populations of tumor cells [28, 29]. Still, given the much greater yield of cells that can be obtained with FACS, this method will most likely provide deeper coverage of the proteome in any given cell type than will LM. FACS coupled with MS-based proteomics has already been achieved for multiple Arabidopsis root cell types as well as mouse colon stem cells [40, 58]. In addition, the INTACT method also promises to be a useful tool for proteomic analyses. While INTACT is limited to proteins in the nucleus, this may actually be advantageous for studies of nuclear processes due to the inherently reduced complexity of the protein sample.

A combination of FACS and MS has been used to measure metabolite concentrations of the auxin indole-3-acetic acid in different cell types of the root [39]. This and other studies suggest that utilizing the appropriate MS platform to analyze extracts of FACS-purified cells will allow a more comprehensive view of the metabolome in individual cell types [9]. While a number of metabolites have been identified in LM-purified vascular bundles [30], currently FACS is likely to be the method of choice for cell type-specific metabolome profiling given the vastly higher cell yields.

4.5 Integrating current methods to provide a comprehensive analysis of individual cell types

The methods described here have already been used to address a range of questions in plant biology, but these studies have generally measured only a single cellular parameter at a time.

The depth of our understanding of any given process could be greatly increased by combining techniques to measure multiple parameters in a given cell type. This would allow analyses of the relationships between the transcriptome, translatome, and proteome, paving the way for studies of post-transcriptional regulation in a given cell type. Comparison of datasets from different experiments has already revealed discrepancies between the transcriptome and translatome [47] as well as between the transcriptome and proteome [40].

Such comprehensive analyses of an individual cell type would be possible by using a combination of INTACT, TRAP, and FACS to collectively examine the genome, epigenome, transcriptome, translatome, proteome, and metabolome of an individual cell type. This could be achieved by creating a single transgenic plant line expressing fluorescent, affinity-tagged nuclear envelope and ribosomal proteins in a specific cell type, which would be a suitable substrate for all of these purification methods (Fig. 2). However, in practice this will be quite labor-intensive, highlighting the need for the development of new methods that are capable of simplifying such endeavors.

5. New technological advances are needed for the analysis of specific cell types

As described above, currently available tools allow examination of DNA, chromatin, RNA, proteins, and metabolites in individual cell types, and these technologies are sufficient to begin creating an atlas of these parameters in many different plant cell types. However, one major limitation is that this requires the extensive use of methods that rely on cell type-specific promoters, and these have not been identified for all cell types and certainly not beyond the main model plant species. Furthermore, FACS, the method with the greatest versatility in terms of parameters it can be used to measure, is not likely to perform well for many cell types outside of the root due to complications from autofluorescent components in other cell types. Thus, it will be critical to optimize current methods and to develop new techniques toward meeting most or all of the following criteria: a) the method does not require specific, *a priori* knowledge of the molecular properties of the targeted cell type, b) transgenic plants are not required, c) it provides high yield and purity of the desired cell type, and d) it can be performed in any laboratory.

Aside from the methods currently in use, we put forward two general ideas that could be pursued as tools for the isolation of specific plant cell types. First, it should be possible to produce antibodies against plant cell surface proteins, some of which will be unique to a particular cell type, and these could be used as affinity purification reagents (Fig. 3A). Secondly, the inherent symmetry of some plant organs means that treatment of a tissue with cell wall-degrading enzymes should hypothetically release one cell layer at a time, and thus specific cell types might be isolated through a digestion time course (Fig. 3B).

The first potential solution could begin by blindly creating a panel of antibodies against plant cell surface proteins and screening these antibodies for those that react specifically with individual cell types in a tissue. Those that do bind specifically could then be used as affinity purification reagents, allowing specific cell types to be quickly isolated from protoplast suspensions (Fig. 3A). Such a panel of antibodies could be generated through the

use of membrane-enriched protein fractions as antigens for the production of monoclonal antibodies. It should be straightforward to generate hundreds of monoclonal antibodies for any plant of interest by using relatively high-throughput methods, such as direct selection of hybridomas (DISH) [59], and some of these antibodies may be useful for multiple species.

This type of approach has been used extensively for the purification of hematopoietic cells [60, 61] as well as for purification of stem cells from mouse embryos [62], supporting the plausibility of such a pursuit. In addition, at least one report describes the isolation of a monoclonal antibody against a plant cell surface protein [63].

A second approach to purifying specific cell types may come by exploiting the symmetry of plant organs. For example, because an anther consists of concentric layers of cells, each containing only one cell type, digestion of an anther into individual cells or protoplasts will hypothetically occur one layer at a time (Fig. 3B). This would create a situation where only one or two cell types are released at a time, and therefore, cells or protoplasts isolated at particular time points will be enriched for specific cell types. If necessary, a second separation step based on a simple size screen or one of the microfluidics-based cell separation strategies that are currently in practice could be employed to purify specific cell types [64, 65]. A similar approach might also be used for other organs consisting of concentric cell layers, such as roots or stem. Even leaves, which have roughly discrete layers of cells, might be amenable to such an approach.

While each of these ideas may seem difficult to realize, they are both firmly based in technologies that are currently in practice. Thus, in trying to move the field of cell purification forward, the obvious challenges posed by the proposed methods should be viewed as areas for testing and optimization rather than roadblocks to prevent further exploration.

6. Conclusions

Compared to comprehensive molecular studies examining whole tissue, only a limited number of studies have been conducted at the resolution of individual cell types. While analyses of whole tissue have illuminated the majority of what is known about plant systems biology, the use of cell-type specific profiling methods is necessary to understand how an organism develops and functions in terms of its composition by specialized cell types. Several useful methods now exist to perform comprehensive molecular analyses of specific cell types, and these include the mechanical cell isolation techniques of LM and FACS as well as the TRAP and INTACT methods for affinity purification of specific cellular components. Each method has one or more molecular parameters that it is particularly useful for measuring. By combining multiple methods to focus on a single cell type, we can gain a comprehensive view of its inner workings and role within a tissue. Current technologies such as FACS, INTACT, and TRAP can provide high yields of purified material to work with, but they are all limited by the requirement for prior knowledge of a promoter active specifically in the cell type of interest and the need to create a transgenic line for each cell type to be studied. On the other hand, LM can be performed on any tissue, but the low yield of material makes certain measurements impractical. Thus, the development of new and

widely applicable approaches will be needed to bring our understanding of plant systems biology down to cell type resolution and to extend our reach beyond model species and into agronomically important ones.

However, beyond technology development there is still much experimental work to be done. Most available global cell-type specific datasets are from single time points or conditions, although cell fate acquisition, development, and responses to the environment are dynamic processes in space and time. Thus, there is a great need not just for cataloging the molecular parameters that characterize each cell type, but also for examining the dynamics of these parameters during development and in response to biotic and abiotic factors in the environment. Furthermore, all of these datasets must be integrated in such a way so as to allow hypotheses to be generated and tested through genetic and molecular biological experimentation. This will require the development of common data processing platforms and organized, accessible storage of these data. In this way it will be possible to understand plant biology at the molecular level and to examine the similarities and differences between divergent plant lineages.

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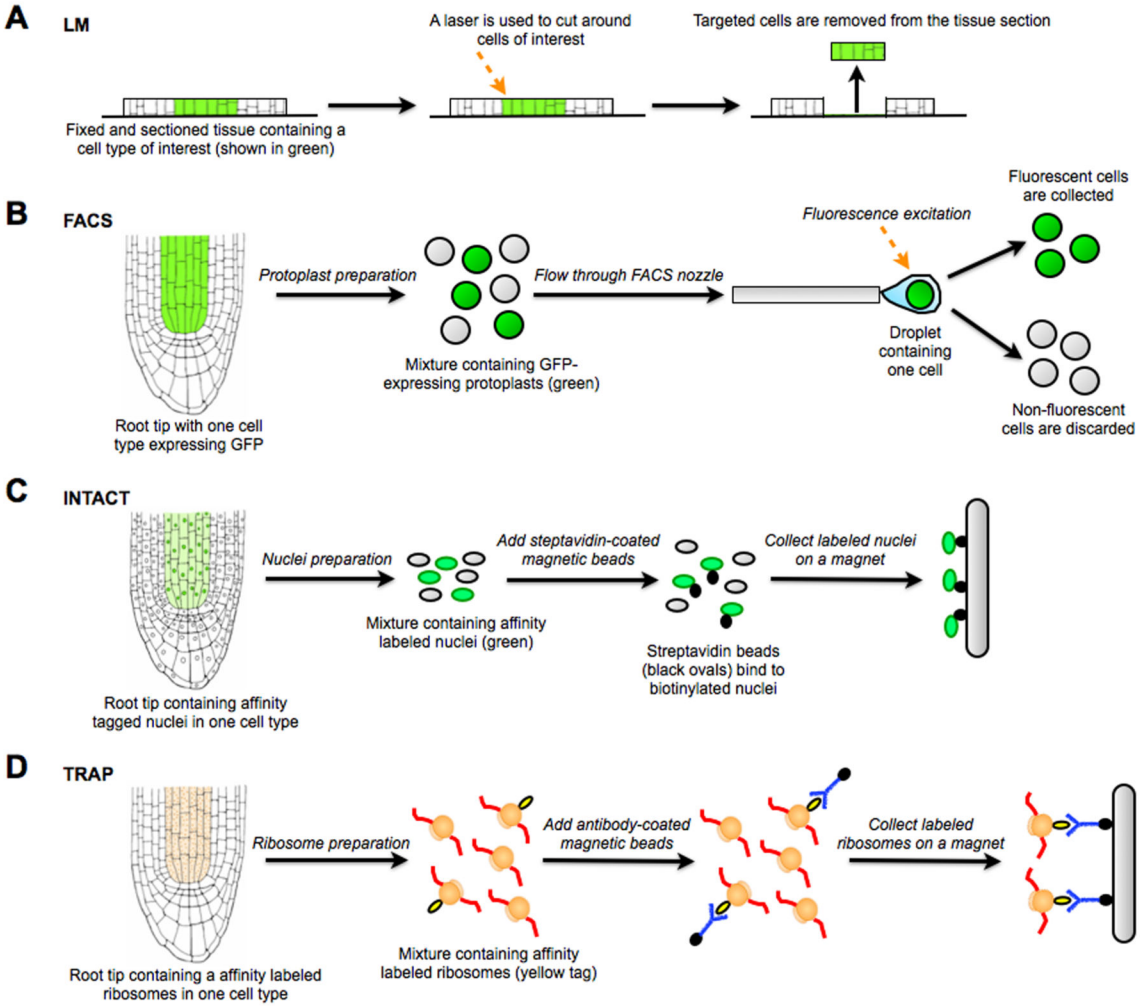

Fig. 1.

Diagrams of the procedures for LM, FACS, INTACT, and TRAP. (A) Laser microdissection (LM); Fixed and sectioned tissue is applied to a slide, and then cells of interest (depicted in green) are excised from the surrounding cells using a laser. The excised cells can now be captured for analysis. (B) Fluorescence-activated cell sorting (FACS); a root tip expressing GFP in a single cell type (depicted in green) is used as an example of the starting material. The tissue is first digested to release protoplasts. Protoplasts are then injected into the FACS system, the nozzle of which produces a stream of droplets, each containing a single cell. A laser is used to excite GFP, and fluorescent droplets are then charged. These charged droplets, containing the cell type of interest, are diverted into a collection tube as they pass by the charged deflecting plates of the FACS sorter. Non-fluorescent cells are discarded. (C) Isolation of nuclei tagged in specific cell types (INTACT); a root tip expressing a biotinylated nuclear envelope protein in a single cell type (depicted in green) is shown as the starting material. A crude nuclei preparation is made by grinding the tissue, and streptavidin-coated magnetic beads are then added to the mixture. Nuclei from the cell type of interest (depicted in green) are then captured on a magnet and nuclei from other cell types are washed away. (D) Tagged ribosome affinity purification (TRAP); a root tip expressing an

epitope-tagged ribosomal protein in a single cell type (depicted in orange) is shown as the starting material. A total ribosome preparation is made, and magnetic beads coated with the epitope-specific antibody are then added. Ribosomes and associated RNAs are then captured on a magnet and ribosomes from other cell types are washed away.

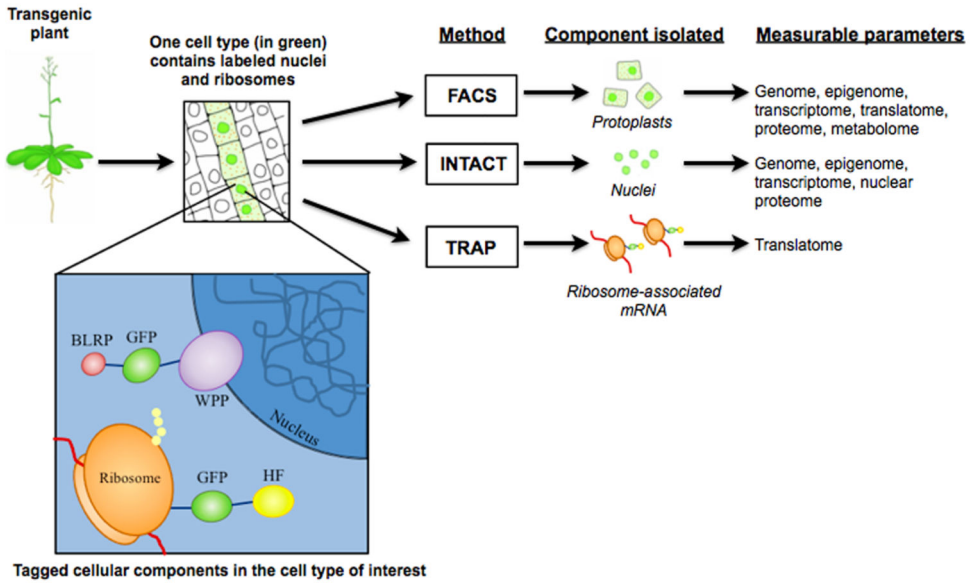

Fig. 2.

Model for integrating multiple cell type-specific profiling methods. The diagram shows an example of how a single transgenic plant could be used as starting material for the FACS, INTACT, and TRAP methods. This could be made possible through the cell type-specific expression of an affinity-tagged nuclear envelope protein and ribosomal protein, both of which also contain GFP. The nuclear envelope tagging protein consists of the nuclear envelope-associating WPP domain of RanGAP (WPP), GFP, and the biotin ligase recognition peptide (BLRP), which is a substrate for biotinylation. The ribosomes of the same cell type would be labeled by expression of ribosomal protein L18 containing GFP as well as 6-His and FLAG tags (HF). Protoplasts of the targeted cell type could be isolated through FACS by virtue of the GFP tag on each protein, and nuclei and ribosomes could also be purified from this cell type by INTACT and TRAP, respectively. The diagram shows the component isolated by each technique and the parameters that could be measured using the purified material.

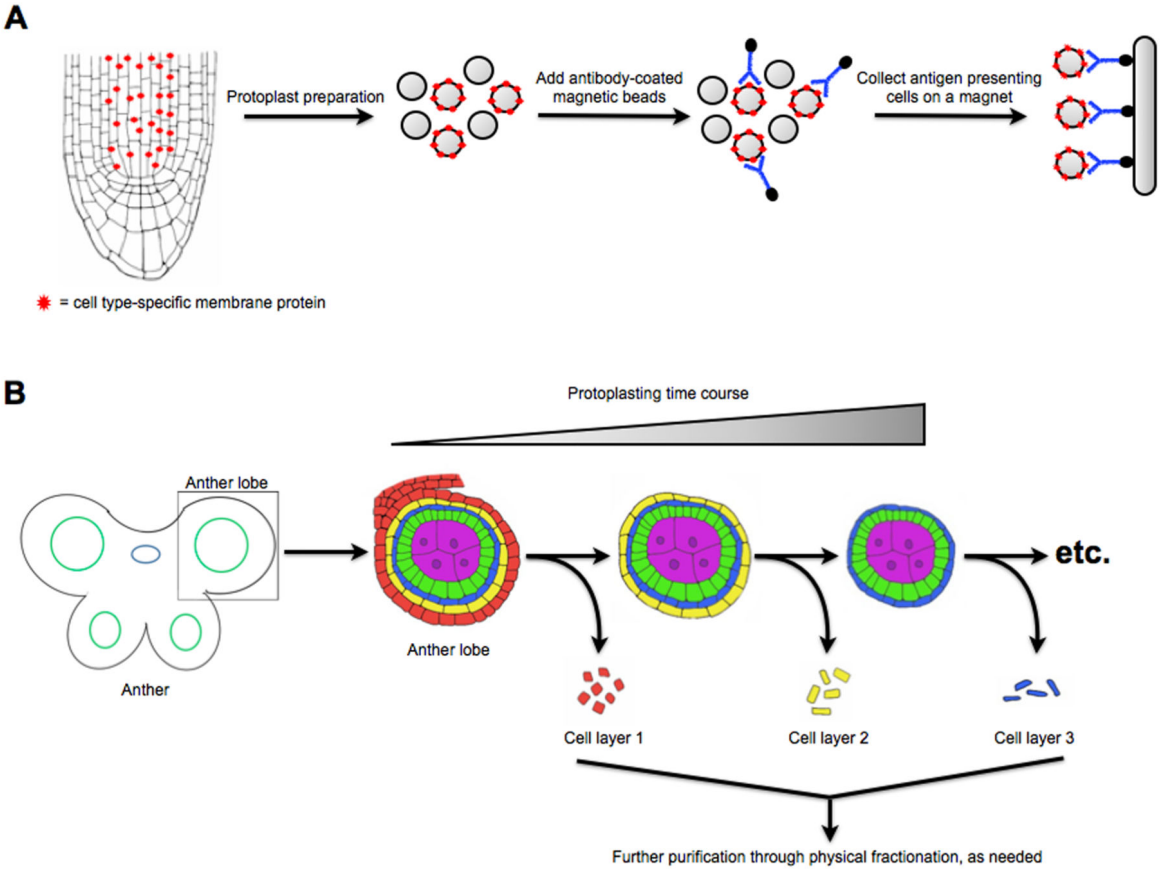

Fig. 3.

Two proposed ideas for the isolation of specific cell types. (A) The first method would use antibodies against a cell type-specific membrane protein to affinity purify the desired cell type. The example shown is a root tip in which an endogenous membrane protein (depicted as a red dot) is found specifically on the surface of one cell type. A protoplast suspension from this tissue would be treated with magnetic beads coated with an antibody against the cell type-specific membrane protein, allowing affinity purification of the desired cell type. We propose that a large collection of antibodies against plant cell surface proteins would provide a toolbox for the isolation of many different cell types using this approach. (B) This hypothetical method would isolate specific cell types from symmetrical organs through sequential release of each cell layer during a time course of treatment with cell wall degrading enzymes. The example shown is an anther lobe, in which each concentric layer of cells consists of one cell type. Treatment of this tissue with cell wall degrading enzymes would release cell layer 1 (red) first, followed by cell layer 2 (yellow), then cell layer 3 (blue), and so on. Optimization of the time course would allow fractions enriched in each cell type to be isolated by aspiration of protoplasts at the appropriate point in the digestion. The protoplasts in solution at any given time are likely to be a low-complexity mixture of cell types, and further purification could be performed as needed. For example, further purification of the desired cell type might be achieved by a simple size selection or by

microfluidic approaches that are able to separate cells on the basis of various physical properties [61, 62].