Single-cell-type Proteomics: Toward a Holistic Understanding of Plant Function*§

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Multicellular organisms such as plants contain different types of cells with specialized functions. Analyzing the protein characteristics of each type of cell will not only reveal specific cell functions, but also enhance understanding of how an organism works. Most plant proteomics studies have focused on using tissues and organs containing a mixture of different cells. Recent single-cell-type proteomics efforts on pollen grains, guard cells, mesophyll cells, root hairs, and trichomes have shown utility. We expect that high resolution proteomic analyses will reveal novel functions in single cells. This review provides an overview of recent developments in plant single-cell-type proteomics. We discuss application of the approach for understanding important cell functions, and we consider the technical challenges of extending the approach to all plant cell types. Finally, we consider the integration of single-cell-type proteomics with transcriptomics and metabolomics with the goal of providing a holistic understanding of plant function. *Molecular & Cellular Proteomics 11: 10.1074/mcp.M112.021550, 1622–1630, 2012.

Plant organs and tissues are composed of various differentiated cells. Each cell type has specific functions in plant growth, development, and interaction with the environment. The analysis of different types of highly specialized cells is essential for understanding the sophisticated molecular networks of regulatory and metabolic pathways underlying plant functions. Most of the functional genomics studies have used entire plant organs or tissues (e.g., leaves, roots, flowers, and seeds) as experimental materials. For instance, proteomics of plant tissues/organs has revealed thousands of proteins in different plant species under different environmental conditions (1). In Arabidopsis and rice, 13,029 and 2528 proteins have been identified, respectively, in various tissues (1, 2). These results are useful in deriving tissue- and organ-specific functions. However, they lack the resolution and selectivity necessary for understanding specific proteins and their functions in different cell types because the information at the cellular level has been diluted and averaged. Therefore, single-cell-type studies are important for unraveling molecular processes underlying the functions of various differentiated cells.

State-of-the-art proteomics technologies have enabled high throughput and sensitive analysis of signaling and metabolic processes in single cells. To date, most reports of single-cell-type proteomic studies are on bacteria, yeast, cultured mammalian cell lines, and red blood cells (3–6) because of the ease of material acquisition. In contrast, there are only a limited number of plant single-cell-type proteomics studies. This situation can be partly attributed to the technical challenges of isolating an adequate quantity and quality of cells from plant tissues. Clearly, dedifferentiated plant cell cultures have advantages, as they contain all the genetic information and are not limited in quantity. Using cell suspension cultures of Arabidopsis, rice (Oryza sativa), tobacco (Nicotiana tabacum), Medicago, and chickpea (Cicer aritinum), a total of 1107, 1528, 360, 1708, and 724 proteins have been identified, respectively (7–12). The data have revealed specific proteins in the secretome (9, 12), extracellular matrix (13), cell wall (14), vacuoles (8), plastids, and peroxisomes (10, 15), as well as abscisic acid- (ABA) (7), temperature- (16), or lipopolysaccharide-treated cell cultures (17) (supplemental Table S1). However, the findings from this model system of cell cultures might not reflect the highly specialized processes and functions of differentiated plant cells. Therefore, proteomics experiments of plant reproductive cells (pollen grains and egg cells) (18–32), specialized leaf epidermal cells (guard cells and trichomes) (33–42), mesophyll cells (35), and root hair cells (43–45) have been conducted (Table I). In some cases, quantitative protein expression changes in the course of cell development and in response to environmental factors have been investigated (24–26, 29–31, 34, 36, 37, 43). The knowledge obtained has enhanced our understanding of specific proteins and cellular events and provided novel insights into the molecular networks and dynamics underlying the functions of specific types of plant cells. In this review, we focus on reviewing the current status and recent development in differentiated single-cell-type plant proteomics. We discuss technical challenges and perspectives in its integration with

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| Cell type   | Species             | Sample/treatment                          | Approaches                  | Protein identities | Unique proteins | Reference |
|------------|---------------------|-------------------------------------------|-----------------------------|--------------------|-----------------|-----------|
| Pollen     | Arabidopsis thaliana| Mature pollen                             | 1DE, ICAT, LC-MS/MS         | 3500                | 3876            | 18        |
|            |                     | Mature and germinated pollen              | 2-DE, MALDI-TOF/TOF MS      | 189                |                 | 19        |
|            |                     | Mature pollen                             | 2-DE, LC-MS/MS             | 135                |                 | 20        |
|            | Lycopersicon esculentum| Mature pollen                            | 2-DE, MALDI-TOF MS         | 121                |                 | 21        |
|            | Oryza sativa        | Mature pollen and coat                    | 2-DE, MALDI-TOF MS, LC-MS/MS| 322                | 401             | 23        |
|            | Lilium davidii      | PM from mature and germinated pollen      | iTRAQ, LC-MS/MS            | 223                | 223             | 25        |
|            | Lilium longiflorum  | Membrane/organelle from pollen tube       | 1-DE, LC-MS/MS             | 270                | 270             | 26        |
| Zea mays   | Pollen coat         | 2-DE, immunoblot                          |                            | 4                  | 4               | 27        |
| Pinus strobus | Pollen tube         | 2-DE, MALDI-TOF MS                        |                            | 38                 | 38              | 28        |
| Pinus bungeana | Pollen tube treated with nifedipine | 2-DE, LC-MS/MS                 |                            | 34                 | 34              | 29        |
| Picea meyeri | Pollen tube treated with trifluoperazine | 2-DE, LC-MS/MS | 93                 | 116              | 30              | 31        |
|            | Pollen tube treated with latrunculin B | 2-DE, LC-MS/MS | 53                 |                  |                 |           |
| Egg cell   | Z. mays             | Egg cell                                  | 1-DE, 2-DE, LC-MS/MS       | 6                  | 6               | 32        |
| Guard cell | A. thaliana         | Guard cell                                | 2-DE, LC, MALDI-TOF/TOF MS, MudPIT | 1734             | 1786           | 33        |
|            | Brassica napus      | v.s. mesophyll cell                       | iTRAQ, LC-MS/MS            | 1458               | 2827           | 35        |
|            | ABA treatment       | iTRAQ, LC-MS/MS                           | 451                         | 34                 |                 |           |
|            | Brassica napus      | v.s. gpa1 mutant                          | iTRAQ, LC-MS/MS            | 1220               | 37              |           |
|            | ABA treatment       | iTRAQ, LC-MS/MS                           | 431                         | 36                 |                 |           |
| Trichome   | A. thaliana         | Non-glandular                             | LC/MS/MS                    | 63                 | 63              | 38        |
| Nicotiana tabacum | Glandular          | 2-DE, 1-DE, LC-MALDI-TOF/TOF MS           | 1538                        | 1543              | 39        |
| Nicotiana tabacum | Glandular          | 2-DE, MALDI-TOF MS, LC-MS/MS              | 7                            | 40                 |                 |           |
| Ocimum basilicum | Glandular          | MudPIT                                    | 881                         | 881                | 41        |
| Solanum lycopersicum | Glandular        | 1-DE, LC-MS/MS                           | 1973                         | 1973               | 42        |
| Root hair  | Glycine max         | Bradyrhizobium japonicum infection        | 2-DE, MALDI-TOF MS, LC-MS/MS| 123                | 1531           | 43        |
|            | Root hair           | Root hair                                 | 1-DE, 2-DE, MALDI-TOF/TOF MS, LC-MS/MS, MudPIT | 1492            | 44              |           |
| Z. mays    | Root hair           | 1-DE, LC-MS/MS                            | 2573                        | 2573               | 45        |

For publications on cell suspension culture proteomics, please refer to supplemental Table S1.
other large-scale “omics” results with the goal of providing a holistic understanding of plant function.

**Status of Plant Single-cell-type Proteomics**—Unlike metazoan systems in which different types of single cells (e.g., blood cells, sperm cells, and lymphocytes) are available, higher plants do not produce free-moving single cells in their life cycle, except pollen grains and spores. Thus, a major bottleneck in plant single-cell proteomics is obtaining different types of cells in adequate quantities and of sufficient quality for proteomic analysis. To date, single-cell-type plant proteomics has been done with reproductive cells (pollen grains and egg cells) (18–32), mesophyll cells (35), and specialized epidermal cells (guard cells, trichomes, and root hairs) (33–45). Multiple complementary gel-based and gel-free proteomic strategies have been utilized in the studies (Table I). The two-dimensional gel electrophoresis (2-DE) approach provides direct visualization of protein molecular weight/isolectric point patterns and inference of protein isoforms and posttranslational modifications (PTMs). Shotgun proteomics approaches include one-dimensional gel electrophoresis liquid chromatography mass spectrometry and multidimensional protein identification technology (MudPIT). These approaches have been used to analyze the proteomes of guard cells, pollen grains, trichomes, and root hairs. For quantitative analysis, technologies involving isotope-coded affinity tags and isobaric tags for relative and absolute quantitation (iTRAQs) have been applied in pollen and guard cell studies. Using the above approaches, a total of 5120 pollen proteins, 4613 guard cell proteins, 4460 trichome proteins, 4104 root hair proteins, 1116 mesophyll cell proteins, and 6 egg cell proteins have been identified. These datasets have revealed specific signaling and metabolic features in each type of functionally specialized cell (Fig. 1).

**Pollen and Egg Cell**—Pollen proteomics results represent the most comprehensive dataset of plant single-cell-type proteomics. Proteomics studies have been carried out with reproductive cells (pollen grains and egg cells) (18–32), mesophyll cells (35), and specialized epidermal cells (guard cells, trichomes, and root hairs) (33–45). Multiple complementary gel-based and gel-free proteomic strategies have been utilized in the studies (Table I). The two-dimensional gel electrophoresis (2-DE) approach provides direct visualization of protein molecular weight/isolectric point patterns and inference of protein isoforms and posttranslational modifications (PTMs). Shotgun proteomics approaches include one-dimensional gel electrophoresis liquid chromatography mass spectrometry and multidimensional protein identification technology (MudPIT). These approaches have been used to analyze the proteomes of guard cells, pollen grains, trichomes, and root hairs. For quantitative analysis, technologies involving isotope-coded affinity tags and isobaric tags for relative and absolute quantitation (iTRAQs) have been applied in pollen and guard cell studies. Using the above approaches, a total of 5120 pollen proteins, 4613 guard cell proteins, 4460 trichome proteins, 4104 root hair proteins, 1116 mesophyll cell proteins, and 6 egg cell proteins have been identified. These datasets have revealed specific signaling and metabolic features in each type of functionally specialized cell (Fig. 1).
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tosis/exocytosis, and cell wall remodeling (29). Similar regu-
tions are skewed toward protein synthesis/folding/modifica-
tion, membrane trafficking, and signal transduction. These 
results are consistent with the recent discovery of lipid raft-
related proteins were found to be associated with the PM. 
This finding implies a special mechanism of facilitating newly 
synthesized proteins for fast pollen tube tip growth (25). The 
result is consistent with the recent discovery of lipid raft-
targeted ribosomal proteins (47). Further analysis of the spa-
tiotemporal dynamics of 270 membrane-related proteins in 
the PM, tonoplast, endoplasmic reticulum membrane, and 
inner mitochondrial membrane revealed that membrane/pro-
tein trafficking and ion transport are active during different 
stages of pollen germination. In rehydrated pollen grains, 
a number of processes were found to be active, including cy-
toskeleton reorganization, carbohydrate/energy metabolism, 
and ion transport. In growing pollen tubes, the protein func-
tions are skewed toward protein synthesis/folding/modifica-
tion, membrane trafficking, and signal transduction. These 
processes are clearly important for pollen tube tip growth (26). 
In gymnosperms, blocking the L-type Ca\(^{2+}\) channels was 
found to affect a series of cytological responses and the 
expression of more than 50 proteins in P. bungeana pollen. 
The protein changes reflect early signaling and energy meta-
bolic events in response to extracellular/cytosolic Ca\(^{2+}\) levels, 
followed by actin filament dynamics, modulation of endocy-
tosis/exocytosis, and cell wall remodeling (29). Similar regul-
atory processes were also observed in P. meyeri pollen grains 
through analysis of the profiles of 93 proteins in different 
stages of pollen germination when the Ca\(^{2+}\)-CaM signaling 
was abrogated (30). These results have linked Ca\(^{2+}\)/CaM 
signaling to the dynamic changes of proteins and pollen tip 
growth.

Unlike free-living pollen grains, the egg cells of higher plants 
are deeply embedded in the ovary and cannot be easily 
isolated. In 1999, a method was developed to isolate maize 
egg cells (48). With this method, the first and only egg cell 
proteomics work identified six abundant proteins: cytosolic 
3-phosphoglycerate kinase, glyceraldehyde-3-phosphate de-
hydrogenase, triosephosphate isomerase, mitochondrial ATP 
synthase β-chain, adenine nucleotide translocator, and an-
nexin P35. Relative to central cells and suspension cells, the 
high expression levels of annexin p35, glyceraldehyde-3-
phosphate dehydrogenase, 3-phosphoglycerate kinase, and 
the adenine nucleotide transporter in the egg cells indicate 
active exocytosis of cell wall materials and energy metabolism 
taking place in the egg cells (32).

Guard Cell—Guard cells (GCs) are highly specialized epi-
dermal cells. They form stomata on the leaf surface. Stomatal 
movement controls plant gas exchange and water transpira-
tion in response to diverse environmental cues. In contrast to 
leaf epidermal cells and photosynthetic mesophyll cells 
(MCs), GCs have distinguished cell structures, such as radially 
oriented cellulose microfibrils and active ion channels in the 
PM and vacuolar membrane, few chloroplasts, and abundant 
mitochondria. The unique structures ensure high energy met-
abolism and active solute transport for flexible cell shape 
change and stomatal movement. The swelling and shrinking 
of GCs can be directly attributed to the influx and efflux of 
various ions (e.g. K\(^+\), Cl\(^-\), and malate\(^{2-}\)). These processes 
are controlled by phytohormone-regulated signaling and meta-
abolic pathways, including Ca\(^{2+}\) oscillation, protein (de)phos-
phorylation, reactive oxygen species (ROS) homeostasis, 
cytoskeleton dynamics, cell wall modification, and photosyn-
thesis/energy supply. Previous molecular and genetics studi-
es have characterized a limited number of proteins in GCs 
(e.g. only 67 proteins in Arabidopsis GCs) (33). Recently, using 
proteomics approaches including 2-DE, two-dimensional LC-
MALDI, MudPIT, and iTRAQ, 1786 and 2827 unique proteins 
were identified in Arabidopsis and Brassica napus GCs, re-
spectively (33, 35). Among the B. napus proteins, 74 were 
quantified as enriched proteins in GCs relative to MCs. Most 
of the proteins were first identified in GCs. Functional analysis 
of the proteins revealed that the GC proteome is skewed 
toward signaling, membrane transport, glycolysis, photosyn-
thesis light reaction, and fatty acid biosynthesis. These func-
tional categories are very different from the functional enrich-
ment of leaf and MC proteomes (Fig. 1). In addition, GC 
proteomics data have corrected some misleading results of 
specific organ marker proteins. For examples, B. napus GC 
proteomics revealed a PM intrinsic protein 2 and a methylthio-
alkylmalate synthase-like protein. The two proteins have been 
thought to be unique markers for roots, which lack GCs (33). 
Using an iTRAQ-based comparative proteomics approach, 
ABA- and methyl jasmonate (MeJA)-responsive proteins in 
GCs from B. napus and Arabidopsis have been identified (34, 
36, 37). In B. napus GCs, 66 ABA-induced and 38 ABA-
decreased proteins were reported to be involved in signaling, Ca\(^{2+}\) oscillation, ROS homeostasis, and photosynthesis/energy supply (36). In Arabidopsis GCs, two proteins in wild-type and six in a G protein \(\alpha\) mutant (\(gpa1–4\)) were affected significantly by ABA treatment (34). Recently, 49 MeJA-induced and 35 MeJA-decreased proteins were discovered in \(B.\ napus\) GCs (37). These results not only confirmed some known GC components, but also revealed at least 10 novel proteins shared in ABA and MeJA signaling pathways. The 186 identified ABA- and/or MeJA-responsive GC proteins have expanded the existing GC hormone signaling network based on about 30 proteins in Arabidopsis GCs (49).

**Trichomes**—Trichomes are highly specialized epidermal cells. They can be classified as either simple trichomes or glandular secreting trichomes. The simple trichomes on Arabidopsis leaves consist of a unicellular structure with a stalk and three to four branches. The glandular trichomes on tobacco leaves have a multicellular structure with a stalk terminating in a glandular head. Trichomes contribute to various aspects of plant defense against biotic and abiotic stresses. They are excellent models for studying cell fate, differentiation, and specialized metabolism. Recently, proteomic analyses led to identification of 1543 proteins in tobacco leaf trichomes (39, 40). The trichome-enriched components represented different enzymes in the synthesis of terpenoid and acyl sugars, diverse stress-responsive proteins, and transporters (39, 40). In addition, proteomic analyses revealed obvious differences in the distribution and accumulation of 881 unique proteins in glandular trichomes from four different sweet basil (\(Ocimum basilicum\) L.) lines. The integration of proteomics results with expressed sequence tags (ESTs)\(^1\) and metabolic profiling data has highlighted the fact that major metabolic branching points controlling the production of phenylpropanoids/terpenoids are regulated by differential gene expression. The chemical diversity of some enzymes in the different basal lines could be attributed to posttranscriptional and posttranslational regulations (41). Especially, the phosphorylation modification of enzymes in the 2-C-methyl-d-erythritol-4-phosphate (MEP)/terpenoid and shikimate/phenylpropanoid pathways is important in metabolic regulation (41).

**Root Hair**—Root hairs are unique tubular cells. They are formed by the differentiation of root epidermal cells by tip growth. Using 2-DE and shotgun proteomics approaches, the dicot species soybean (\(G.\ max\)) and monocot species maize (\(Z.\ mays\)) root hair proteome reference maps (1531 proteins and 2573 proteins, respectively) have been established (43–45). The proteins provide useful insight into the molecular processes of signaling, transcription, protein degradation, energy production, lipid metabolism, specialized metabolism, hormone action, and cell wall dynamics in root hair polar growth and water/nutrient uptake (44). It should be noted that legume root hairs are the primary sites for rhizobial infection leading to the formation of nodules. The nodules can reduce the conversion of atmospheric nitrogen into ammonia for plant nutrition. Comparative proteomic analysis identified a number of root hair specific proteins and \(Bradyrhizobium japonicum\) responsive proteins in soybeans (43). These novel proteins suggest that lipid signaling, cytoskeleton dynamics, starch metabolism, and lectin production play important roles in root hair nodulation (43).

**Improved Understanding of Cellular Processes and Functions**—

**Signal Transduction and Membrane Trafficking/Transport**—Pollen tubes, root hairs, and trichomes are polar growing cells. They constantly interact with environmental cues. In mature pollen grains from rice and Arabidopsis, some pre-synthesized signaling proteins for tube tip growth have been found to be enriched (18, 23). For example, nearly half of the small guanosine triphosphate hydrolases (GTPases) and one third of the SNAREs have been identified in Arabidopsis mature pollen grains for active signaling and vesicle transport in growing pollen tubes (18). Quantitative proteomics of germinating pollen grains from Arabidopsis, rice, lily, and gymnosperm species has been conducted. The results revealed that proteins in the G-protein signaling pathway, \(Ca^{2+}\) signaling proteins, ion transport/channel proteins, components in clathrin-dependent endocytosis and vesicle trafficking pathways, and PM-localized kinases exhibit dynamic changes upon pollen germination (19, 24, 25, 29–31). These proteins highlight integrated signaling cascades and vesicle trafficking for pollen tip growth (Fig. 1). However, many of these proteins have not been found in proteomics studies of root hairs and trichomes. This is probably because the focus of the studies is on environmental effects instead of root hair/trichome development from epidermal cells. For example, in soybean root hairs inoculated with \(B.\ japonicum\), the activated phospholipase D (PLD) in lipid signaling was supposed to function in growing pollen tubes (41). Other signaling proteins (e.g. 14–3-3 proteins, annexins, and G proteins) might function in response to biotic and abiotic stresses (44). In Arabidopsis and tobacco trichomes, a 14–3-3 protein and a glutathione S-conjugate ABC transporter were hypothesized to function in plant detoxification (38, 40).

As for GCs, signaling and membrane transport proteins are mainly responsible for stomatal movement. In Arabidopsis GCs, 52 proteins were classified as signaling proteins, which were highly represented by various G proteins, PLDs, and protein kinases (33). Some candidate proteins in \(Ca^{2+}\) signaling cascades (e.g. PLD and early response to dehydration (ERD) protein 14) and the ABA signaling pathway (e.g. cation/\(H^+\)-exchanger 20, OPEN STOMATA 1, and myrosinases

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1 The abbreviations used are: EST, expressed sequence tag; GC, guard cell; iTRAQ, isobaric tag for relative and absolute quantitation; MC, mesophyll cell; MeJA, methyl jasmonate; PM, plasma membrane; PTM, posttranslational modification; ROS, reactive oxygen species.
TGG1 and TGG2) were discovered in GCs from wild-type Arabidopsis and a gpa1–4 mutant using iTRAQ-based proteomics (34). Similarly, some ABA-responsive signaling proteins (e.g. 14–3–3, MAPK, cytokinin binding protein, calmodulin, protein phosphatase 2A) were found to be highly abundant in B. napus GCs relative to MCs (36). In addition, a Rab GDP dissociation inhibitor was induced under ABA treatment (36). All these findings have contributed to a better understanding of ABA-regulated molecular networks in stomatic GCs.

**Cytoskeleton Dynamics and Cell Wall Remodeling**—Pollen proteomic analyses suggest that some key cytoskeletal and cell wall proteins are pre-synthesized in mature pollen grains. In germinating pollen tubes of rice, Arabidopsis, P. meyeri, and P. bungeana, important cytoskeletal proteins such as actin, tubulin, actin depolymerizing factor, actin binding protein, myosin, and profilin exhibited significant changes in expression (19, 24, 29–31). The protein changes contribute to the observed cytoskeleton dynamics in pollen grains. Interestingly, the expression profiles of cytoskeletal proteins in P. meyeri and P. bungeana pollen tubes were found to be responsive to germination inhibitors and extracellular Ca\(^{2+}\) (29, 30). The data suggest that the dynamic changes of actin microfilaments and motor proteins control the cytoplasmic reorganization and the delivery of secretory/endocytic vesicles during pollen tube elongation. Some of the cytoskeletal components (e.g. tubulins and actins) were also detected in the proteomes of soybean root hairs and B. napus GCs (35, 44). Tubulin 5 in GCs and actin in root hairs were induced by ABA and B. japonicum infection, respectively (36, 43). The results suggest the importance of cytoskeleton dynamics in stomatic movement and root nodulation.

Cell-wall-metabolism-related proteins were found to be highly represented in pollen tubes. In germinating rice and P. strobos pollen grains, cell wall proteins with changed expression account for 25% and 7% of the identified proteins, respectively (24, 28). Cell wall metabolic proteins are represented by UDP-glucose pyrophosphorylase, UDP-glucose dehydrogenase, expansin, and reversibly glycosylated polypeptide (RGP). They showed significant changes after treatment with germination inhibitors or extracellular Ca\(^{2+}\) (29, 30). The proteomics data have shown that the pollen cell wall undergoes dramatic remodeling upon pollen germination. In addition, at least 41 proteins were found to be released from the pollen coat and/or interior and possibly bound to the stigma surface. The released proteins included various enzymes involved in carbohydrate and energy metabolism, cell wall loosening, and pollen allergens. They are supposed to function in the interaction between pollen and stigma and the hydrolysis of transmitting tract cells in stigma (24). In contrast, only four cell wall remodeling proteins (cellulose synthases, endo-1,4-β-glucanase, xyloglucan endotransglycosylase, and pectinesterase) were identified in soybean root hairs (44). In GCs that undergo rapid shape changes for stomatal movement, three cell wall metabolism-related proteins (UDP-β-glucose/UDP-β-galactose 4-epimerase 1, RGP, and polysaccharide synthase) were clearly enriched relative to MCs (35). In summary, cytoskeleton dynamics and cell wall remodeling are important processes in different types of cells during development and interaction with environmental cues. The differential representation of proteins in these functional categories in different cell types is an interesting observation.

**ROS Scavenging and Redox Regulation**—ROS are known to play important roles in pollen tube growth (50), root hair development (51), stomatal movement (37), and trichome stress response (52). Redox proteins identified in single-cell-type proteomics studies suggest that multiple ROS scavenging pathways are important in quenching ROS generated in signal transduction and/or in oxidative stress. The following scavenger proteins have been found to display dynamic changes upon pollen germination: ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR), and legumin in rice pollen (24); APX and cyclophilin in P. meyeri pollen (30, 31); and peroxiredoxin (PrxR), glutathione S-transferase (GST), glutathione peroxidase (GPX), monodehydroascorbate reductase, and APX in Arabidopsis pollen (19). This implies that the glutathione-ascorbate cycle, PrxR/thioredoxin (Trx) pathway, and GPX pathway are triggered in pollen tubes of different species to maintain ROS and redox homeostasis and to protect from oxidative damage during rapid tube tip growth. The expression of some of the scavengers (e.g. GST, late embryogenesis abundant proteins (LEA), cyclophilin, and APX) were also found to be significantly changed in soybean root hairs after infection by B. japonicum. In addition, different antioxidative enzymes, including superoxide dismutase (SOD), PrxR/Trx, GPX, GST, and glutaredoxin, were identified in trichomes from tobacco (39, 40) and Arabidopsis (38). Together with other defense-related proteins (e.g. glucanases, chitinases, endochitinase, phylloplanins, and glutathione S-conjugate translocator) identified in the trichome proteomes (38, 39), the trichome enriched proteins provide strong molecular evidence for the hypothesis that trichomes are involved in defense against stresses caused by xenobiotics and fungal infection (53). The function of ROS in GCs has been well studied. For example, H\(_2\)O\(_2\) serves as a secondary messenger of GC hormone signaling to regulate Ca\(^{2+}\) homeostasis, protein (de)phosphorylation, channel activities, and stomatal movement (37). Recent proteomics results revealed that some of the ROS scavenging enzymes (e.g. DHAR, Prx, ERD, and APX) were overrepressed in GCs relative to MCs. Most of these enzymes were activated by ABA (34, 36) or MeJA (37). In ABA-treated B. napus GCs, the expression levels of APX, GPX, GST, cyclophilin, Prx, and Trx showed significant changes (36). In MeJA-treated GCs, seven ROS-related enzymes (two isoforms of Fe-SOD, Prx, APX, GST, glycolate oxidase, and germin-like protein) and other antioxidants (such as LEA and lipoygenase) were induced (37). The functions of ROS in different cell types appeared to vary.
Future studies exploring how ROS regulate downstream processes and relate to specific physiological functions in different cells will be exciting.

Carbohydrate/Energy Metabolism and Specialized Metabolism—In the proteomes of pollen grains and pollen tubes of Arabidopsis and rice, proteins involved in carbohydrate/energy metabolism and protein synthesis/fate are overrepresented (19–20, 23–24). These proteins are essential for starch degradation and sucrose synthesis to supply carbon skeletons and energy for rapid protein synthesis and proper folding in the course of pollen tube tip growth. In B. napus GCs, carbohydrate/energy metabolism and protein metabolism-related proteins account for at least 40% of the preferentially expressed proteins. In MCs, photosynthesis-related proteins constituted over 50% of the proteins (35). This reflects the functional differentiation of GCs for stomatal movement and MCs for photosynthesis (Fig. 1). Interestingly, proteomics of Arabidopsis GCs detected thioglucoside glucohydrolase (TGG) 1 (a.k.a. myrosinase) as a highly abundant protein in GCs. It specifically catalyzes glucosinolate hydrolysis to form isothiocyanates and other degradation products (33). In B. napus GCs, a TGG2 homolog and myrosinase-associated proteins were found to be induced by ABA (36). Further functional analysis using tgg1 and tgg2 mutants demonstrated the importance of myrosinase in inward K⁺ channel regulation during ABA inhibition of the stomatal opening (33). Recently, the glucosinolate degradation product allyl isothiocyanate has been shown to induce stomatal closure in Arabidopsis via ROS and nitric oxide production (54). These results have demonstrated a novel function of specialized glucosinolate metabolism that had previously been associated with defense roles against pathogens and herbivores (55). It is now also clearly important in GC ABA signaling and stomatal movement. In glandular trichomes of tobacco and basil, primary metabolism and energy-related proteins take up more than 40% of the proteome, but many proteins have been found to function in specialized metabolism (39–41). For instance, all the enzymes in the MEP pathway, some terpenoid-related enzymes, ABC transporters, and lipid transfer protein 1 are present at high levels in the trichomes. With sufficient carbon and energy supply, the enhanced specific metabolic activities in the glandular trichomes enable the synthesis and secretion of a diverse spectrum of chemicals for plant defense against pathogens and insects. These results exemplify how proteomics studies can shed light on the signaling and metabolic networks of specific types of cells.

Current Challenges and Perspectives—In recent years, single-cell-type transcriptomics studies have been advancing very fast in areas of suspension cell cultures (7–17), germinating pollen (56), trichomes (57), root hairs (58), and GCs (59). In addition to transcriptional control of plant processes, it is now clear that there are multiple posttranscriptional levels of control. For example, many enzymes are regulated by PTMs and metabolic control. Such information is difficult or impossible to glean from genomic studies. Here we focus on recent advances made in specialized single-cell-type proteomics.

Unlike with genomics, the following challenges and problems of single-cell-type plant proteomics constitute major limitations: sampling sufficient amounts of specific single-cell-type materials with minimal contamination; low proteome coverage of scarce proteins (no PCR types of technologies available for proteins); capturing accurate quantitative changes of proteins, modifications, interactions, and activities; limited sequence databases for non-model species; and software capability, data throughput, and sharing. With continuing developments in laser capture microdissection, cell sorting, mass spectrometers, genome sequencing technologies, and proteomic informatics, these challenges are not insurmountable. For example, fluorescence activated cell sorting technology can greatly enhance the purity and homogeneity of different cell types (60). Top-down proteomics and targeted multiple reaction monitoring mass spectrometry are likely to achieve 100% proteome coverage in the future. Such capabilities will not only lead to the construction of whole genome scale single-cell-type proteome databases, but also enable ultra-high sensitive proteomic analysis on a single cell.

In yeast, quantitative proteomics has been applied in the systematic elucidation of the galactose utilization network. Many proteins that might be subject to PTMs were identified, and several unexpected regulatory mechanisms were revealed (4). In E. coli, multiple large-scale analyses (including proteomics and metabolomics) of wild-type and its variety of metabolic mutants have revealed novel connections, fluxes, and a robust metabolic network (3). Research in plants has started to move in the direction of integrative analysis. For example, analysis of ESTs/cDNAs, proteins, and metabolites in sweet basil (O. basilicum) and tomato (S. lycopersicum) glandular trichomes led to the discovery of some specific regulatory pathways and metabolic branching points (41, 42). These studies have demonstrated the power of capturing complex information and relationships through different global measurements. In this review, we have described the utility of plant proteomics approaches in discovering novel proteins, processes, and pathways and in linking protein functions to specific cellular phenotypes. Because of the success in microorganisms and the availability of transcriptomics and metabolomics tools and results from single-cell-type analyses, future research needs to integrate data and parameters across genomics, transcriptomics, proteomics, and metabolomics levels of systemic description. That will ultimately lead to a comprehensive understanding of molecular networks in single cells.

CONCLUSIONS

In recent years, functional genomics technologies have moved beyond simple cataloging toward large-scale molecular quantification and network analysis. Although transcriptomics work has been conducted using different types of
cells, the transcriptional-level data do not directly reflect molecular processes at the protein and metabolite levels. Despite the impressive progress made in the identification and quantification of proteins in different types of cells, the proteomic coverage is mostly limited to relatively abundant proteins. Future studies need to extend the coverage to low abundant proteins (toward 100% proteome coverage) and include protein PTMs (e.g. phosphorylation and redox modification), protein interaction and complex analysis, protein activity and turnover, and organelle proteome dynamics. Metabolomics analyses of some single cell types have been reported lately (61, 62). With the rapid developments in powerful analytical technologies, including mass spectrometry, we can expect a plethora of protein and metabolite data. The grand challenges are to validate and make sense of the data, put molecular components and dynamic changes into particular pathways, integrate with other types of data, and connect into molecular networks of plant cell functions. A systemic knowledge of how cells work will certainly aid our efforts toward obtaining a holistic understanding of plant function.

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