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Quantitative Proteomics in Plants: Choices in Abundance

The field of proteomics is evolving from cataloguing proteins under static conditions to comparative analyses. Defining proteins that change in abundance, form, location, or activity may indicate which proteins are involved in developmental changes or responses to alterations in environmental conditions. Such studies are facilitated by an increasing number of complementary technical options for performing quantitative proteomic comparisons. As with any developing field, however, rapid expansion in new techniques introduces concerns about choosing the appropriate approach. The goals of this perspective essay, therefore, are both to introduce the various options that are available (or nearly so) for quantitative proteomics and to discuss considerations related to applying these methods in the laboratory.

OVERVIEW

Proteomics is well into its second decade as a field of study, so referring to proteomics as a new or recent area of science no longer seems applicable. However, the majority of the first decade was dominated by two-dimensional gel electrophoresis and traditional protein staining techniques (described below) as the primary means of performing comparative experiments. Two-dimensional gels coupled with conventional staining methods have been (and continue to be) productive in providing relevant information about biological systems. However, problems with sensitivity, throughput, and reproducibility of this method place limitations on comparative proteomic studies. The advent of a number of powerful and complementary technologies for performing quantitative comparisons greatly expands the depth and breadth of reliable information that can be obtained about the dynamic proteome. Therefore, the goals of this essay are to provide an overview of these new technologies and to serve as a starting point for those seeking to incorporate quantitative proteomics into their research programs.

The space limitations of this essay preclude a comprehensive description of the many applications of proteomics in plant biology, and we apologize to all authors whose work was not cited. For similar reasons, we cannot discuss all the nuances of each method, and we refer interested readers to a recent book (Samaj and Thelen, 2007; and collection of review articles in the Journal of Experimental Biology [2006], volume 57) for a more in-depth coverage of various methods or applications of proteomics in plant biology.

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TWO-DIMENSIONAL GEL ELECTROPHORESIS

Protein isoelectric focusing coupled to SDS-PAGE, usually referred to as two-dimensional gel electrophoresis (2-DE), is a common procedure to resolve proteins based upon native charge followed by mass. Protein separation based upon these two unrelated properties can produce impressive, complex maps of proteins. However, the reproducibility of 2-DE gels can be problematic due to the diverse properties of proteins. In addition to problems with technical reproducibility, matching of 2-DE spots amongst a group of gels can be an arduous task. When performing comparative proteomics using 2-DE, it is routinely necessary to analyze multiple gels containing $>$1000 spots each. Even with advanced 2-DE analysis software, such analyses are challenging because the highest accuracy requires final manual validation of each computationally generated spot group, a cohort of matched 2-DE spots (Hajduch et al., 2006). To simplify this task and maximize accuracy of spot matching, high-quality 2-DE gels with minimal spot streaking and overlap are critical. Other imperfections with 2-DE gels are related to sample overloading that occurs as one tries to increase the percentage of the proteome that can be visualized. Ultrasensitive, quantitative detection methods, such as fluorescent dyes (e.g., Sypro Ruby [Invitrogen] and Deep Purple [GE Healthcare]), attempt to address this sample loading concern (Miller et al., 2006).

One technique that addresses the issues of both sensitivity and gel variability is difference gel electrophoresis (DIGE). DIGE involves preincubating protein samples with activated fluorescent dyes to label Lys (or Cys) residues with a sensitive tag that can be used to quantify the abundance of that protein in solution (Tonge et al., 2001). When performed with charge-matched (in the case of Lys reactivity) reactive dyes, the labeled protein will migrate to the same isoelectric position on a 2-DE gel as the unlabeled protein and produce similar 2-DE reference maps as traditional staining methods, although slight mass shifts are possible below 20 kD.

The obvious advantage to prelabeling of proteins with spectrally distinct fluorescent tags is the ability to combine protein samples to be separated within the same gel. The ability to analyze multiple samples in a single 2-DE gel greatly simplifies spot matching and quantification such that most 2-DE analysis software can excel at this task. In theory, sample multiplexing is limited only by the number of fluorescence-emitting dyes with nonoverlapping spectral patterns. However, only three different charge-matched, Lys-reactive dyes are commercially available at present (Cy2, 3, and 5; GE Healthcare). The current manufacturer's recommendations are to employ Cy2 as the internal

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control if multiple DIGE gels are performed (Alban et al., 2003). Once resolved in the gel, the labeled proteins are visualized using a laser fluorescent scanner capable of acquiring two sets of data (i.e., Cy3 and Cy5) in a single scan (e.g., Typhoon or EDI Imager [GE Healthcare] or FLA5000 [Fuji Medical]). The high molar absorptivity of Cy dyes enables the routine detection of >1000 protein spots with only 50 μg of protein sample. Thus, the detection of pico- to femtomolar levels of protein is achievable with this approach.

Unfortunately, the ultrahigh sensitivity of DIGE is matched by only a handful of commercial mass spectrometers. Thus, although one can profile proteins at the subpicomolar levels using DIGE, direct protein identification from these gels can be challenging. One way to overcome this is to resolve a separate, preparative 2-DE gel containing the Cy-labeled protein samples added to larger amounts of unlabeled protein. After fluorescent imaging, this preparative gel is overstained with Coomassie blue such that the spots of interest can be matched between the fluorescent and Coomassie images and excised for mass spectral analysis.

From budgetary and bioinformatics standpoints, another upside to DIGE is the ability to focus downstream mass spectrometry efforts on only those differentially expressed or posttranslationally modified protein spots. Therefore, DIGE is highly appropriate for comparative profiling of knockout, transgenic, or isogenic germplasm as well as defined pharmacological or stress-induced responses as recent reports suggest (Casati et al., 2005; Amme et al., 2006; Mooney et al., 2006; Hjerno et al., 2006; Hajduch et al., 2007; Keeler et al., 2007; Bohler et al., 2007). Another important consideration is that quantitative studies using 2-D gels in general, and 2-D DIGE in particular, can be performed with any plant species and are not restricted to plants with sequenced genomes. For example, Hajduch et al. (2007) employed a 2-DE DIGE strategy to compare near-isogenic sunflower lines varying in achene oil content. These authors outlined a novel statistical strategy for determination of differential expression. As expected for near-isogenic lines, a small percentage of the total 2-DE spots were differential. Many of the differential proteins were involved in glycolysis and protein synthesis or storage, supporting a relationship between oil and protein content. In another study, Bohler et al. (2007) employed 2-DE DIGE to analyze the effect of ozone on protein expression in poplar leaves. The results from this study supported previous observations of reduced ribulose-1,5-bis-phosphate carboxylase/oxygenase activase and increased glycolytic activities in response to ozone stress.

Experiments in which few changes in protein expression or posttranslational modification are expected are ideally suited for the DIGE approach. In addition, the requirement of only a laser imager and analysis software to perform DIGE may make this a more affordable approach for quantitative proteomics compared with mass spectrometry–based quantitative approaches (see below). It should be emphasized that despite these advantages, DIGE suffers from the same problems as traditional 2-DE, including underrepresentation of extreme proteins, such as proteins with high/low molecular weights or extreme isoelectric points as well as hydrophobic membrane proteins.

QUANTITATION BY MASS SPECTROMETRY

Alternatives to 2-DE gel-based quantification of intact proteins are mass spectrometry (MS)–based approaches that compare the abundance of peptides as surrogates for intact proteins. Although a variety of mass spectrometers exist with differences in how they detect and fragment peptides (reviewed in Domon and Aebersold, 2005), MS analyses have a similar general work flow. After digestion of proteins with a protease (typically trypsin), the complex peptide mixture is separated by chromatography either directly coupled to the mass spectrometer (online separation) or prior to MS analysis (off-line). The peptides are ionized to acquire the initial MS scan, a spectrum of the mass-to-charge ratio of peptide ions in that sample. Selected peptides from the MS scan are then individually fragmented for the MS/MS (or MS2) scan to collect amino acid sequence information about the peptides.

Signal or peak integration of ions in the MS scans has been used as a quantification technique for decades by small molecule analytical chemists because theoretically the peak intensity of any ion should be proportional to its abundance. However, technical variation, both at the liquid chromatography and ionization stages, might render comparisons of peak intensities between experiments unreliable. The recent advent of label-free quantitative methods suggests these issues may not be as great a concern as once thought (see section on label-free quantitation below). Nonetheless, the desire to avoid variations between runs using different samples was the basis for developing labeling strategies that would allow direct comparisons of peaks (corresponding to the peptide abundance in different samples) within the same MS or MS/MS scan. At a basic level, these strategies are variations on a similar theme: inert, stable, isotopic mass tags are introduced to the peptides such that the ionization and chromatographic properties of the tagged peptides are similar but the sample origin (e.g., from treatment A or treatment B) can be deciphered after analysis based upon a signature mass shift either in the MS or MS/MS spectrum. Mass separation and subsequent quantification of the ion current for these peptide mass pairs or peptide groups reflects the relative abundance of that peptide, a surrogate for the abundance of the intact protein from which the peptide was derived. The main differences between these labeling methods are when the tag is introduced into the protein/peptide and how the quantitative data are extracted (summarized in Table 1).

IN VIVO ISOTOPIC LABELING

In vivo metabolic labeling of proteins with isotopes is a common method for comparative proteomics. In this experimental design, one set of samples is grown on a natural nitrogen source while the comparative sample is grown in the presence of the heavy

Table 1. A Comparison of Labeling Methods for MS-Based Quantitative Proteomics

Label Method	In Vivo	In Vitro	MS Mode	MS/MS Mode	Used in Plants
^{15}N metabolic labeling	×		×		Ippel et al. (2004); Whitelegge (2004); Whitelegge et al. (2004); Engelsberger et al. (2006); Benschop et al. (2007); Huttlin et al. (2007)
SILAC	×		×		Gruhler et al. (2005)
^{18}O-trypsin		×	×		Nelson et al. (2006)
ICAT		×	×		Islam et al. (2003); Dunkley et al. (2004); Majeran et al. (2005); Hartman et al. (2007)
iTRAQ		×		×	Dunkley et al. (2006); Jones et al. (2006); Rudella et al. (2006); Nühse et al. (2007); Patterson et al. (2007)
Label-free			×	×	Chen et al. (2005) (2007); Majeran et al. (2005); Niittyla et al. (2007)
AQUA		×	×		Glinski et al. (2003); Glinski and Weckwerth (2005)

isotope. The isotopic label can be introduced either as an amino acid (termed stable isotopic labeling) in cell culture (SILAC) or by ^{15}N as the sole nitrogen source, typically in the form of $K^{15}NO_3$. Although differences in these labeling methods affect the complexity of the analysis, as discussed below, the end result in both cases is that the masses of peptides from the two or three populations will be different, allowing for a direct comparison of MS peak intensities between the two samples. In theory, the differential samples can be mixed very early in the experiment, virtually eliminating potential variation that might arise from technical variation during subcellular fractionation or chromatographic separation. A limitation, however, is that only two or three samples can generally be compared at one time, limited both by the ability to introduce distinguishing isotopic tags into the cells and by the resulting increase in complexity in the MS scan. Also, because all peptides in the MS scan are not always sequenced, high mass accuracy in the MS mode is necessary to ensure that the peaks being compared are the same peptide sequence and not one with a very similar mass. Finally, because the isotopic difference equates to two MS peaks for each peptide, a full proteome study (i.e., all soluble proteins) can be problematic because of the complexity of the MS spectrum. Therefore, isotopic labeling generally is better suited for comparisons of subproteomes, such as phosphopeptides (Benschop et al., 2007).

Stable Isotopic Labeling by Amino Acids in Cell Culture

In general, SILAC has the advantage of a simpler analysis compared with metabolic labeling with ^{15}N. Usually, a single amino acid is used for SILAC. If the supplied amino acid is Lys or Arg, analysis of peptides from a trypsin digest that cleaves after these two amino acids will result in peptides containing only a single difference from the labeled amino acid. Therefore, the mass difference between peptides in the MS scan will be known and consistent. However, because plants can synthesis all amino acids from inorganic nitrogen, the labeling efficiency achieved using exogenous amino acid feeding of *Arabidopsis* cell cultures has been found to average only 70 to 80% (Gruhler et al., 2005). The result is that a portion of the peptide from the heavy isotopic-labeled sample is not labeled and will always contaminate the unlabeled peptide, complicating the analysis and thus requiring reciprocal labeling. The other disadvantage of SILAC is that the labeled amino acids are expensive when used in amounts needed for efficient labeling, so this method is likely to be limited to plant cell cultures. However, suspension-cultured cells are responsive to a number of stimuli. For instance, Gruhler et al. (2005) used this approach to identify specific subsets of glutathione *S*-transferases and 14-3-3 proteins that were differentially regulated in response to treatment with salicylic acid.

^{15}N Metabolic Labeling

By contrast with SILAC, metabolic labeling with ^{15}N has been shown to achieve >98% incorporation in both intact plants (Ippel et al., 2004) and cell cultures (Engelsberger et al., 2006) and is more cost effective than SILAC. The trade-off is that because all amino acids will incorporate the label, the mass difference between labeled and unlabeled peptides will vary depending on their sequence. Therefore, for each comparison, the peptide must be sequenced before the location of the paired MS peak for the comparative peptide can be calculated and located. Although software performs these tasks, comparisons of highly complex samples can be extremely complicated. An additional consideration is whether all plant tissues can be labeled to a similarly high efficiency with this method. However, recent studies indicate that only partial metabolic labeling in plants may prove effective for comparative studies (Whitelegge et al., 2004; Huttlin et al., 2007).

In Vitro Isotopic Labeling of Peptides

An alternative approach to in vivo labeling is to introduce the label into the peptides chemically during or after digestion of the proteins. One of the main advantages of in vitro labeling of peptides is that the experimental use of source tissues is not limited by the ability to introduce the label to the living cell. Of course, the clear disadvantage is that much greater care is needed to control technical variation introduced during the isolation of protein (e.g., during subcellular fractionation).

^{18}O-Labeling during Trypsin Digest

During hydrolysis of proteins by trypsin, oxygen originating from water in the solvent is incorporated into the C terminus of the

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tryptic peptide. Therefore, performing the trypsin digest of one sample in $H_2{}^{16}O$ and the other in $H_2{}^{18}O$ theoretically results in peptides differing by four mass units in the MS scan (Stewart et al., 2001; Yao et al., 2001). Experimentally, however, the incorporation is rarely complete, resulting in complex overlapping MS spectra. The end result is that the potential problems described for SILAC above are generally exaggerated. In addition, the nature of incorporating the label limits this method to pairwise comparisons. Because of these difficulties, ^{18}O-labeling is not used as often as the other methods described below. However, the relatively low costs of reagents make this a viable option when cost constraints are an important factor in experimental design.

Isotope-Coded Affinity Tags

Another method for labeling peptides after proteolytic digest relies on covalent modification of Cys residues with chemically identical biotinylated tags that differ only in mass because of inclusion of heavy and light isotopes. The use of biotin allows rapid enrichment of the tagged peptides, and because most proteins only contain a few Cys residues, only a few peptides from each protein will be enriched/analyzed. Therefore, this method has the potential to deal with pairwise comparisons of highly complex samples. However, approximately one in seven proteins do not contain Cys residues, guaranteeing limitations in the completeness of the analysis. In addition, posttranslational modification analysis will be limited to only modified peptides containing Cys residues, greatly reducing the value of isotope-coded affinity tags (ICATs) for these types of experiments.

A recent study with proteins from solubilized mitochondria of *Arabidopsis* made use of ICAT to detect potential protein complexes without a priori knowledge of proteins involved (Hartman et al., 2007). Solubilized, native proteins were sedimented through a continuous gradient by ultracentrifugation. Theoretically, proteins should be distributed with monomers near the top and larger protein complexes near the bottom. Proteins from each fraction were digested with trypsin and labeled with one of the ICAT reagents (e.g., heavy isotope). The other ICAT reagent (e.g., light isotope) labeled a pooled control of alternating fractions from the total gradient for relative comparison. The end result was a distribution pattern for each protein within the gradient such that proteins with nearly identical distribution patterns were candidates for being together within a complex.

Isobaric Tags for Relative and Absolute Quantitation

An alternative method involves chemical modification of primary amines with isobaric tags for relative and absolute quantitation (iTRAQ) (Ross et al., 2004). The experimental design is similar to that used for ICAT in that each sample receives its own unique tag, but iTRAQ reagents label (nearly) all tryptic peptides instead of only those containing Cys residues. In addition, iTRAQ allows comparison of up to four samples in the same experiment (new 8-plex reagents are set to be released by Applied Biosystems in 2008). Another difference is that quantitation occurs in the MS/MS scan after fragmentation of the peptide. Peptide fragmentation releases the iTRAQ mass reporter of 114, 115, 116, or 117 D, and the intensity of this reporter peak reflects the relative quantity of the peptide in each sample. Because the comparison occurs in the MS/MS scan (i.e., when the peptide is sequenced), relative quantitative comparisons are obtained unambiguously for each peptide sequenced.

The possibility of labeling at least four samples allows analysis of time-course experiments or to perform biological replicates in a single analysis (i.e., internally repeat control versus treatment experiments). Both applications were used in conjunction with chromatographic enrichment of phosphopeptides to identify proteins undergoing differential phosphorylation in response to microbial elicitation of *Arabidopsis* suspension-cultured cells (Nühse et al., 2007). Another application in plants was to use iTRAQ reagents to obtain quantitative information from regions of 2-D gels for which spots had not resolved sufficiently to provide unequivocal results (Rudella et al., 2006). The goal of that study was to investigate proteomic changes in the chloroplasts of *clpr2-1*, a mutant of a plastidial protease complex. The ClpP/R/S subunit proteins of the Clp core complex ran as overlapping spots on 2-D gels, obscuring accurate quantitation, but isolation of this complex gel region followed by iTRAQ labeling of peptides from gels containing control and mutant protein samples demonstrated that most of the subunits of the Clp complex were decreased in the mutant but to different degrees.

LABEL-FREE QUANTITATION

As discussed in the introduction to this section, the signal intensity of peptide ions within an MS scan can be compared en mass from multiple liquid chromatography–mass spectrometry (LC-MS) analyses. This peak integration method is referred to as label-free quantification because no isotopic label is introduced into the proteins or peptides. Although this approach is still in its relative infancy, the reproducibility of online chromatographic separation of peptides combined with the high mass accuracy of the latest generation of mass spectrometers machines offers renewed promise for this method.

Various software programs have been developed to match peptides from multiple raw LC-MS/MS files using combinations of retention time and precursor mass characteristics to iteratively match peptides that elute from a typical liquid chromatography gradient. Once matched, the peak areas corresponding to the matched peptides (from the extracted ion chromatogram) are compared to arrive at an expression ratio. Although these software programs are new, the concept of comparing ion chromatogram signal intensities is not. These new programs merely perform this task in a high-throughput, systematic manner using powerful statistics, including recursive base peak monitoring to arrive at a series of pairwise group expression assignments.

An alternative form of label-free quantification is spectral counting. Unlike peak integration, which calculates peak ion intensity from MS scans, spectral counting tabulates the number of MS/MS scans that are attributed to the same precursor ion (i.e., peptide). The frequency of these MS/MS scans (in theory) reflects the abundance of this peptide in the sample. Spectral counting is an approach that appeals to another developing characteristic of contemporary mass spectrometers: speed of data acquisition. For example, if 10 scans can be acquired per second on a mass spectrometer, a 2-h analytical gradient would yield >50,000 MS/MS scans, assuming two of the 10 scans are MS acquisitions. This information from a simple LC-MS/MS run represents an unmined reservoir of expression data comparable in number to EST DNA sequence reads from a cDNA library screen. However, at this point, it is unclear whether dynamic exclusion rules frequently applied during mass spectral acquisitions invalidate the spectral counting approach. Dynamic exclusion is used to maximize the number of peptides sequenced during tandem MS acquisitions. Individual peptides elute from a reversed-phase analytical column in the time scale of minutes, while a mass spectrometer collects data on the second or millisecond scale. Therefore, rather than constantly resequencing an abundant peptide, dynamic exclusion can be applied to ignore ions for which MS/MS spectra have already been acquired. Typically, the duration of this exclusion is ~30 s.

A recent study comparing peak integration and spectral counting found these two label-free methods to be in general agreement, with indications that spectral counting was more sensitive, whereas peak integration was more accurate (Old et al., 2005). These methods were used to examine changes in the proteome of mammalian cell lines treated with phorbol esters, which stimulate a mitogen-activated protein kinase pathway leading to cell attachment and spreading. The results for proteins identified as changing in abundance during the treatment were in agreement with changes in transcript abundance for these proteins as determined by microarray analysis, confirming the validity of these proteomics analyses.

ABSOLUTE QUANTIFICATION USING AQUA PEPTIDES

All quantitative proteomics methods discussed up to this point are capable of determining the relative abundance of proteins or peptides. Relative quantification approaches are suitable for most experiments in which the objective is to discover differentially expressed or modified proteins. Converting relative quantification data to absolute quantitative levels requires the inclusion of internal standards of known concentrations. The internal protein or peptide standards must be labeled to distinguish them from the in vivo, native protein or peptide and, similar to the other methods described above, stable isotopic labeling of peptides is the preferred strategy.

Synthetic peptides with a heavy amino acid at one or more positions are termed AQUA peptides in reference to their use for absolute quantification, as reported by Gerber et al. (2003). Since the chromatographic and ionization properties of AQUA peptides are identical to their cognate native peptide, by analyzing and integrating the extracted ion chromatogram for the isotope pair in the MS scan, one can obtain a ratio of the native peptide to the AQUA peptide standard of known concentration. Similarly, a specific concentration of standard peptide could be labeled with one of the iTRAQ reagents for absolute quantitation in the MS/MS scan. Conceivably, AQUA peptides could be synthesized to multiple proteins of interest and used to determine the absolute levels of these proteins in a sample or to address their stoichiometry. An additional area of research that should be particularly well served by the use of AQUA peptides is the quantification of posttranslational modifications (Glinski et al., 2003; Glinski and Weckwerth, 2005).

CONSIDERATIONS FOR INITIATING QUANTITATIVE PROTEOMIC EXPERIMENTS

When faced with a wide assortment of technical options, as is the case with quantitative proteomics, an obvious question arises: Which is the best method? The current consensus across the field is that no strategy is clearly superior to another in all cases. Studies in which direct comparisons were made using different methods found that when experiments were designed and performed properly, the technical variation of the various methods was comparable, and the resulting data generally showed good agreement between methods (Kolkman et al., 2004; Choe et al., 2005; Wu et al., 2006). In plants, few direct comparisons of methods are available, although there is no reason to believe the conclusion would be different. One of the only published cross-comparisons of methods in plants used 2-D gels, ICAT, and label-free quantitative methods to analyze differential accumulation of maize chloroplast proteins in bundle sheath versus mesophyll cells (Majeran et al., 2005). Of the 125 proteins quantified in these experiments, only 20 proteins were quantified by all three methods, demonstrating the complementary nature of pursuing multiple strategies. In other cases, different quantitative methods have been successfully employed to investigate related biological questions. Experiments isolating compartments of the endomembrane system were performed successfully using either ICAT (Dunkley et al., 2004) or iTRAQ (Dunkley et al., 2006). Similarly, studies investigating changes in protein phosphorylation under various treatment conditions or developmental stages in plants have been performed using 2-D gels (Shin et al., 2007), in vivo metabolic labeling (Benschop et al., 2007), iTRAQ (Nühse et al., 2007), and label-free methods (Niittyla et al., 2007). Therefore, multiple methods are capable of providing important new insights into plant biology.

It is important to acknowledge, however, that the technical variation for each method will be influenced by the individual performing the experiment and the precision of the equipment. Therefore, although each method is capable of success, each laboratory must determine technical variation within its own experimental environment rather than refer to the statistical

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robustness of the method as published elsewhere. Indeed, voluntary analysis of a blind sample distributed by the Association of Biomolecular Research Facilities concluded that choice of method was less important than the laboratory's experience with the chosen method (Turck et al., 2007). In addition to technical variation, a successful quantitative proteomic experiment must also take into consideration biological variation. Biological variation arises in part from the inability to grow, stage, dissect, or isolate biological samples in a perfectly consistent manner. It is imperative that researchers recognize that technical reproducibility does not replace the need for biological replication as would be required in any other experimental design. As was found during the standardization of microarray experiments, biological replication is an essential component of statistically meaningful comparative studies (Nettleton, 2006), and these same principals should be applied to proteomic studies. Therefore, publication of proteomic data should include clear distinctions between biological and technical repetitions when describing how often an experiment was repeated. Moreover, the experimental design must work within financial constraints to allow for sufficient biological and technical replicates to generate meaningful conclusions.

When deciding upon a quantitative proteomic method to employ, a major consideration will be the resources available to the researcher, particularly in regards to the type of mass spectrometer. All of the stable isotope quantification approaches minimally require a mass spectrometer capable of obtaining isotopic resolution. Label-free quantitative experiments will require many days of uninterrupted access to the mass spectrometer for analyzing samples in tandem to avoid variation in the system, something which may be difficult to arrange if the work is conducted in a proteomics facility. By contrast, 2-D gel analyses are less reliant on mass spectrometers or other specialized equipment (e.g., DIGE requires only an isoelectric focusing unit and a dual-channel imager). Postanalysis bioinformatics and statistics are other factors to consider when choosing a quantitative proteomics approach. Analysis of SILAC, ICAT, or iTRAQ data without appropriate software to detect and quantify the mass tags can be a frustrating endeavor. Cross-compatibility of extant commercial or open-source software with the type of mass spectrometer frequently must be empirically determined. Therefore, one must consider the entire work flow, from sample isolation to statistical analysis, when deciding on which quantitative method is accessible to one's laboratory.

SUMMARY

The ability to compare dynamic changes in the proteome is an exciting new addition to the research programs of many plant biologists. With alternative transcription/translation and the potential addition of over 200 different posttranslational modifications to proteins, the complexity of the proteome is likely to exceed the complexity of the transcriptome by one to two orders of magnitude, making the proteome as vast and complex as it is dynamic. A variety of options for performing quantitative proteomic comparisons in plants is available and currently in use by a number of laboratories. As we hope we have emphasized, presently no single method is more highly preferred over another. However, neither will any single method provide a complete overview of all the changes in a proteome. This admission is something that should simply be accepted rather than serve as a deterrent from initiating proteomic studies. Any quantitative proteomic method can yield new insights into the biological system, regardless of whether some information has been missed. With some of these quantitative methods beginning to reach technical maturity, we look forward to comparative proteomic studies moving out of the realm of technical experts and spreading throughout the community of biological researchers.

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CURRENT PERSPECTIVE ESSAY

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