PCP
PLANT & CELL PHYSIOLOGY

# Protein–Protein Interactions in Plants

Yoichiro Fukao*

Plant Global Educational Project, Nara Institute of Science and Technology, Ikoma, 630-0192 Japan
*Corresponding author: E-mail, fukao@bs.naist.jp; Fax: +81-743-72-6251.
(Received December 12, 2011; Accepted February 21, 2012)

Review

**The study of protein–protein interactions (PPIs) is essential to uncover unknown functions of proteins at the molecular level and to gain insight into complex cellular networks. Affinity purification and mass spectrometry (AP-MS), yeast two-hybrid, imaging approaches and numerous diverse databases have been developed as strategies to analyze PPIs. The past decade has seen an increase in the number of identified proteins with the development of MS and large-scale proteome analyses. Consequently, the false-positive protein identification rate has also increased. Therefore, the general consensus is to confirm PPI data using one or more independent approaches for an accurate evaluation. Furthermore, identifying minor PPIs is fundamental for understanding the functions of transient interactions and low-abundance proteins. Besides establishing PPI methodologies, we are now seeing the development of new methods and/or improvements in existing methods, which involve identifying minor proteins by MS, multidimensional protein identification technology or OFFGEL electrophoresis analyses, one-shot analysis with a long column or filter-aided sample preparation methods. These advanced techniques should allow thousands of proteins to be identified, whereas in-depth proteomic methods should permit the identification of transient binding or PPIs with weak affinity. Here, the current status of PPI analysis is reviewed and some advanced techniques are discussed briefly along with future challenges for plant proteomics.**

**Keywords:** Affinity purification • Database • Mass spectrometry • Protein–protein interaction • Stoichiometry.

**Abbreviations:** AP-MS, affinity purification and mass spectrometry; AQUA, absolute quantitation; BiFC, bimolecular fluorescence complementation; BN-PAGE, blue native-PAGE; CCB, Coomassie Brilliant Blue; DSP, di-thiobis-succinimidyl-propionate; FASP, filter-aided sample preparation; GFP, green fluorescent protein; HA, hemagglutinin; His, histidine; LC, liquid chromatography; MS, mass spectrometry; MudPIT, multidimensional protein identification technology; NPC, nuclear pore complex; Nup, nucleoporin; PPI, protein–protein interaction; RFP, red fluorescent protein; TAP, tandem affinity purification; Y2H, yeast two-hybrid.

## Introduction

The availability of the complete genome sequences of several model organisms (Arabidopsis Genome Initiative 2000, Goff et al. 2002, Yu et al. 2002, International Rice Genome Sequencing Project 2005) has made proteomics an important tool for understanding various plant physiological phenomena at the molecular level (Agrawal and Rakwal 2008). During the early days of plant proteomics, organ and organelle proteomes were studied by applying the rapidly developing techniques of mass spectrometry (MS) analyses coupled with one-dimensional gel electrophoresis (commonly referred to as SDS–PAGE) or two-dimensional gel electrophoresis to reduce sample complexity (van Wijk 2001, Warnock et al. 2004, Agrawal et al. 2011a). Alternatively, separation by HPLC of trypsin-digested peptides in solution and their direct identification by MS as a shotgun analysis has allowed the identification of complex peptide mixtures (Wolters et al. 2001, Wienkoop and Weckwerth 2006, Haynes and Roberts 2007). Besides their application to study the whole plant or plant organ development, proteomic techniques have been widely exploited to investigate biotic or abiotic stress responses in plants (Jorrín et al. 2007, Baginsky 2009, Agrawal et al. 2011a, Kosová et al. 2011). More recently, substantial improvements in MS and other technical advances related to proteomics have permitted various functional analyses such as the identification of post-translational modifications and protein quantification in a wide range of organisms. These aspects have been recently reviewed (Ahrens et al. 2010, Cox and Mann 2011, Nakagami et al. 2011) and therefore will not be dealt with in this review. Here, the current state, limitations and future directions of the protein–protein interaction (PPI) approach in plant biology is presented and discussed.

Studies on PPIs are critical for understanding the functions of proteins of particular interest. To date, various methodologies, including the yeast two-hybrid (Y2H) system, affinity purification and mass spectrometry (AP-MS) analyses, fluorescence imaging such as fluorescence resonance energy transfer and bimolecular fluorescence complementation (BiFC), computational analysis and protein microarray have been developed to detect previously uncharacterized or novel PPIs. The Y2H system has been widely used to examine PPIs in a pairwise manner (Fields and Song 1989, Uetz et al. 2000).

*Plant Cell Physiol.* 53(4): 617–625 (2012) doi:10.1093/pcp/pcs026, available online at www.pcp.oxfordjournals.org

More recently, large-scale PPI analyses have been applied to various organisms, including plants (Parrish et al. 2006, Schoonheim et al. 2007, Tardif et al. 2007, Suter et al. 2008, Yu et al. 2008, Bonetta 2010, Arabidopsis Interactome Mapping Consortium 2011, Seo et al. 2011, Vernoux et al. 2011). However, each of them as a single approach has certain limitations, e.g. when the expressed protein is toxic to the organism in which it is being produced. Moreover, most of these methods are not applicable to the expression and study of large multimeric protein complexes. Thus, several improvements have been developed to overcome these problems (for a review see Zhang et al. 2010). The Y2H approach in plants has been summarized in several excellent reviews (Uhrig 2006, Lalonde et al. 2008, Morsy et al. 2008, Zhang et al. 2010) and will not be covered in this review.

In contrast, AP-MS analysis represents one of the major strategies for PPI analyses. Target proteins fused with FLAG, c-Myc, histidine (His) and hemagglutinin (HA) are used for one-step affinity purification (Chang 2006). The tandem affinity purification (TAP) method was also developed as a two-step affinity purification process to reduce the false-positive rate (Zhang et al. 2010). AP-MS approaches are advantageous for identifying the endogenous state of protein complexes composed of two or more proteins. However, purifying a protein complex is sometimes difficult, because the methodology for extracting a specific protein complex is often not suitable for others. For example, some proteins are often dissociated from the protein complex, degraded and aggregated during the extraction procedure. Therefore, an efficient purification method has to be established for each protein. In addition, AP-MS approaches have high false-positive rates even with use of the TAP method (Zhang et al. 2010). Thus, it has been recommended to perform AP-MS in combination with other methods for PPI analyses. Besides the Y2H and AP-MS approaches, several methodologies for PPI analysis including imaging and database searching approaches have been recently established (Shoemaker and Panchenko 2007, Okumoto 2010, Sadanandom and Napier 2010, Mochida and Shinozaki 2011). The combination of PPI analyses should be useful for producing informative data with high accuracy. This review should be useful not only for plant proteomics researchers but also for plant biologists in general.

## Tandem affinity purification

The TAP method was developed as a major strategy for AP-MS analysis (**Table 1**). In this method, protein complexes are purified from cell extracts in two consecutive steps utilizing two different affinity tags (Li 2011). This method has been widely used for PPI analysis in various organisms, because a much cleaner protein complex is obtained as compared with the one-step affinity purification (Xu et al. 2010). The TAP-tag, which consists of the immunoglobulin G-binding domains of protein A from *Staphylococcus aureus*, the *Tobacco etch virus* protease and calmodulin-binding peptide, was originally developed in yeast (Rigaut et al. 1999, Gavin et al. 2002). Thereafter, the TAP-tag was successfully applied to purify glucocorticoid receptor-interacting proteins (Rohila et al. 2004) and the mutiprotein COP9 signalosome complex (Rubio et al. 2005) from plants. So far, the TAP method has been used to identify PPIs related to disease resistance (Xing and Chen 2006), the cell cycle (Van Leene et al. 2007), mitochondrial biogenesis (Van Aken et al. 2007), salt stress tolerance (Batelli et al. 2007), nucleocytoplasmic transport (Zhao et al. 2008), post-embryonic development (Xing et al. 2008a, Xing et al. 2008b), a protein phosphatase inhibitor (Templeton et al. 2011) in *Arabidopsis thaliana*, and a protein kinase (Rohila et al. 2006, Rohila et al. 2009) and photoperiodic flowering (Abe et al. 2008) in rice. In recent years, there has been an increase in the number of studies on PPIs that improve the TAP method to optimize plant systems with the aim to overcome the relatively low yield compared with that of yeast (Van Leene et al. 2008, Li 2011).

In contrast, low-abundant proteins, such as those expressed at low copy numbers per cell, are sometimes less detectable using the TAP method due to the decreased yield of the target protein complex following the two-step purification process. Moreover, the molecular size of the TAP-tag is largely dependent on a combination of tags, and that could be one cause for the increased number of false-positive proteins bound to TAP-tags (Li 2011). Therefore, increasing the amount of starting material (plant sample) for PPI analysis and purification might ultimately result in false-positive proteins. To overcome this problem, AP-MS methods in conjunction with cross-linking could be useful for identifying low-abundant or low-affinity proteins. In one study, RPS2

**Table 1** Summary of PPI analytical techniques

| Method | Advantage | Disadvantage |
|---|---|---|
| AP-MS | Identification of endogenous PPIs | Optimization of protein extraction method for each protein |
| Y2H | Screening thousands of PPIs including weak or transient interactions | Toxicity of expressed proteins to yeast or unsuitable condition for some plant proteins |
| BN-PAGE | Identification of endogenous protein complexes | Difficulties during protein sample preparation |
| Fluorescence imaging | Spatial and temporal PPI information in living cells | PPIs of exogenous proteins |
| Computational analysis | PPI database based on experimental data | Including predicted PPI data |

(a membrane-associated disease resistance protein), localized on the plasma membrane in low abundance, was purified with or without the cross-linking reagent di-thiobis-succinimidyl-propionate (DSP) followed by the identification of the interacting proteins using the AP-MS method (Qi and Katagiri 2009). Greater amounts of the known RPS2-interacting protein RIN4 were purified with DSP than without DSP. These results suggested that cross-linking reagents might be useful for purifying interacting proteins with membrane proteins or transient binding proteins, because of their stabilizing effect on the protein complex during purification.

## GFP affinity tag purification

Several basic affinity tags such as FLAG, c-Myc, His and HA have been employed for AP-MS analysis as one-step affinity tags (Stevens 2000, Terpe 2003, Cho et al. 2006, Ueda et al. 2011). Green fluorescent protein (GFP) has been utilized as a one-step affinity tag for vacuolar protein sorting 29 (Jaillais et al. 2007) and PIN-FORMED 1 (PIN1), which has transmembrane regions (Mravec et al. 2011) in *A. thaliana*. Tamura et al. (2010) recently developed an AP-MS method for identifying the nuclear pore complex (NPC) using *A. thaliana* plants expressing GFP-fused nucleoporins (Nups), which are components of the NPC. In this method, extracted proteins are incubated with magnetic beads conjugated to an anti-GFP antibody for only 10 min, because with a shorter purification step there will be a high possibility to purify protein complexes in their original state while reducing the false-positive rate (Cristea et al. 2005). The purified PPIs are then eluted with 0.1 M $Na_2CO_3$ and neutralized with 1 M MES for in-solution digestion or a sample buffer containing 4% (w/v) SDS for in-gel digestion after separation by SDS–PAGE. The in-solution digestion method has fewer sample preparation steps for MS analysis. The eluted fraction is digested by trypsin, desalted impurities are removed by acetone precipitation or C18 resin to avoid interference with peptide ionizations, and then the solution is directly analyzed by MS, i.e. only one shotgun analysis is performed per sample. However, protein sample losses might occur during the desalting step, particularly with lower yielding fractions. In contrast, the in-gel digestion method does not require a desalting step prior to SDS–PAGE, because the impurities included in the samples migrate faster than proteins during electrophoresis (Fukao et al. 2009). This also means that impurities do not interfere with peptide ionizations during MS analysis. Nonetheless, some proteins cannot be identified even when enough protein is available due to low peptide coverage from the SDS–polyacrylamide gel. In addition, proteins of extremely high and low molecular weights are less easily detectable because of differences in the rate of entry and movement within the acrylamide gel. In the AP-MS analysis of GFP-fused RAE1, seven and five Nups were identified by in-gel digestion and in-solution digestion, respectively, and 13 Nups were commonly identified by both methods (**Fig. 1**). These results suggest that the rate of protein identification depends on the type of method used and that using two methods can be complementary. Finally, it might be better to conduct the AP-MS analysis using a suitable method or both depending on the experimental purpose.

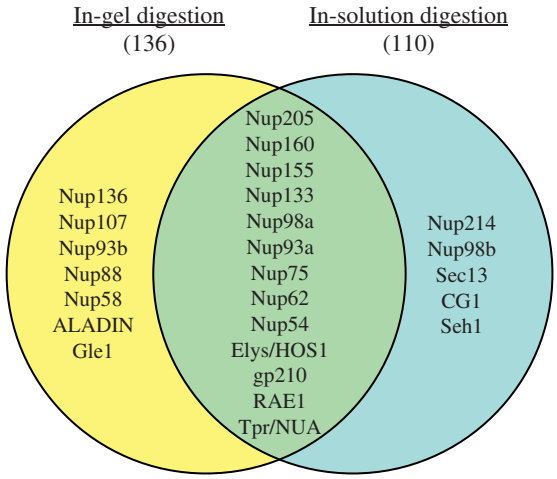


**Fig. 1** Comparison of identified nucleoporins (Nups) between in-gel digestion and in-solution digestion methods. The Nups were identified as follows: seven by in-gel digestion (yellow circles), five by in-solution digestion (blue circles) and 13 by both methods (merged region), respectively. The numbers in parentheses show the total numbers of identified proteins in each method.

Transgenic plants expressing GFP-tagged protein are often constructed to analyze the protein locations (Brandizzi et al. 2002). Complementation of mutant phenotypes by expressing GFP-fused proteins is a good indication of the adequate localization, although we have to consider mislocalization, misfolding and misassembly (Millar et al. 2009, Moore and Murphy 2009). In addition to GFP and its variants such as yellow fluorescent protein and cyan fluorescent protein (Cristea et al. 2005), magnetic beads conjugated to an anti-red fluorescent protein (RFP) antibody also work well for AP-MS analysis (personal communication). The putative interacting proteins detected by the GFP-tag method are often confirmed for co-localization by RFP in a transgenic plant expressing the GFP-fused protein. Therefore, this transgenic system is also available for the next AP-MS analysis using the RFP-tag. The fluorescent protein tag purification method is useful in protein localization and PPI analyses, saving time.

In contrast to the above application, a GFP-tag may increase the false-positive rate due to a larger molecular size as in the case of the TAP-tag, thus making it harder to detect and identify endogenous interacting proteins (Stevens 2000). Therefore, it is my recommendation to use other kinds of basic affinity tags such as FLAG, c-Myc, His and HA in parallel with GFP and then narrow down the commonly identified proteins.

## Large-scale PPI analysis using multiple approaches

As mentioned above, TAP and GFP-tag affinity approaches have their own advantages and disadvantages (**Table 1**); thus, there is no ideal strategy for identifying proteins using a single method (Millar et al. 2009). A systematic large-scale PPI analysis by more than one approach is required. To identify PPIs between core cell cycle proteins, Boruc et al. (2010) recently conducted a large-scale analysis of Y2H and BiFC as two independent complementary interaction assays. To evaluate the quality of the data, they compared the resulting interactions with literature-based data and the results of TAP purification (Boruc et al. 2010, Van Leene et al. 2011). In another study, mating-based split ubiquitin systems were developed as a high-throughput screening method for identifying PPIs between membrane proteins and the interface with signaling proteins (Lalonde et al. 2010). In their work, they tested 490 *A. thaliana* open reading frames including receptor-like kinases, transporters, soluble protein kinases and phosphatases, glycosyltransferases and proteins of other or unknown functions. A total of 343 PPIs among 179 proteins were detected among the 90,370 possible PPIs (Lalonde et al. 2010). For example, the interaction between the ammonium transporter AMT1;1 and two receptor-like kinases was independently confirmed by the split luciferase complementation assay (Kato et al. 2010). These high-throughput analyses have high potential for identifying complex protein networks in plant cells.

In addition to the above approaches, classical biochemical techniques are also applicable to PPI analysis (Miernyk and Thelen 2008, Peng et al. 2009). Native PPIs can be analyzed by blue native-PAGE (BN-PAGE) or a gel filtration chromatography (**Table 1**; Eubel et al. 2005, Klodmann et al. 2011) techniques. In BN-PAGE, the solubilized protein samples obtained by non-ionic detergents are incubated with Coomassie Brilliant Blue (CBB) G250 anionic dye immediately before electrophoresis, and then protein complexes of approximately MegaDalton order can be separated and visualized by BN-PAGE. The target protein complex is also detectable by immunoblotting, CBB staining, silver staining or fluorescence dye (Kang et al. 2011, Suzuki et al. 2011). BN-PAGE has been the main analytical approach for identifying mitochondrial and chloroplast protein complexes in plants, and this approach has recently been extended to the plasma membrane and microsomal fraction (Eubel et al. 2005). Furthermore, organelle proteomics data have been used effectively to evaluate results of PPI analyses (Lilley and Dupree 2007, Agrawal et al. 2011a). Several decades ago, organelle isolation methods were established to determine proteins related to metabolic pathways and component protein profiles of organelles and to measure enzymatic activities to understand organelle functions. Therefore, organelle proteomics was conducted according to the development of MS based on previously established methods, and now several data sets of each organelle are available (for an in-depth review, see Agrawal et al. 2011a). These data sets can be utilized to examine whether the target protein and its interacting partner are localized within the same organelle.

## Database analysis

Besides experimental approaches, the importance of computational analysis is garnering attention and support with the ever-increasing amount of PPI data (**Table 1**; Mochida and Shinozaki 2011). Geisler-Lee et al. (2007) predicted ~20,000 PPIs in *A. thaliana* from a homologous PPI database in *Saccharomyces cerevisiae*, *Caenorhabditis elegans*, *Drosophila melanogaster* and *Homo sapiens*. Furthermore, many databases have been constructed to better evaluate PPI data derived from various experiments: TAIR (Swarbreck et al. 2008; http://www.arabidopsis.org/portals/proteome/proteinInteract.jsp), AtPID (Li et al. 2011; http://www.megabionet.org/atpid/), AtPIN (Brandão et al. 2009; http://bioinfo.esalq.usp.br/atpin/), BioGRID (Stark et al. 2011; http://thebiogrid.org/) and PAIR (Lin et al. 2011a, Lin et al. 2011b; http://www.cls.zju.edu.cn/pair/) specialized to *A. thaliana* or several organisms including *A. thaliana*, and PRIN (Gu et al. 2011; http://bis.zju.edu.cn/prin/) specialized to rice. As for BioGRID, which was originally built based on PPI data from *S. cerevisae* (Breitkreutz et al. 2003), the authors have currently developed a PPI database for several model organisms including *A. thaliana* (Stark et al. 2011). This database contains ~300,000 non-redundant PPI data in version 3.1. It is now possible to obtain the entire data set free and create custom interaction data sets. PAIR has built a resource of predicted *A. thaliana* PPIs and currently contains ~150,000 non-redundant PPI data points, and the predicted PPIs in version 3.3 are expected to cover ~25% of all *A. thaliana* PPIs (Lin et al. 2009). In addition, PRIN is the first database for the rice PPI network, which was built based on PPI data from *S. cerevisiae*, *C. elegans*, *D. melanogaster*, *H. sapiens*, *Escherichia coli K12* and *A. thaliana* (Gu et al. 2011). PRIN currently contains ~80,000 non-redundant rice PPIs among approximately 5,000 rice proteins. These databases are frequently updated, and thus are useful, up-to-date tools for evaluating PPI data.

## Stoichiometry of the protein complex

Quantification methods for endogenous proteomes in planta have been established in various studies (Bindschedler and Cramer 2011). Among them, the multireaction monitoring/selected reaction monitoring method has been recently applied to plants (Lehmann et al. 2008, Zulak et al. 2009). Stable isotope-labeled peptide, also termed the absolute quantitation (AQUA) peptide, allows for absolute protein quantification as an internal standard (Brun et al. 2009). This method is effective for distinguishing and quantifying highly conserved isoforms (Lehmann et al. 2008) and quantifying proteins, such as those in allergens, when the absolute amount of protein must be considered (Stevenson et al. 2010). It is also possible to apply

this approach to non-model plants when there is some information about the amino acid sequences in the target proteins (Zulak et al. 2009). AQUA has been successfully applied to quantify plant membrane proteins, such as major transporters belonging to $H^+$-ATPase AHA, aquaporin PIP, AMT and the nitrate transporter NRT families (Monneuse et al. 2011).

Recent developments have allowed the quantitative analysis of protein complexes by AP-MS analysis (Sharon and Robinson 2007, Heck 2008, Kaake et al. 2010, Schmidt et al. 2010). However, stoichiometry analyses within protein complexes remain a challenge because of poor stability, which is probably affected by solute condition and contamination by false-positive proteins during purification. Recently, Olinares et al. (2011) successfully applied the QconCAT method to determine the subunit stoichiometry of the affinity-purified chloroplast ClpP/R protease in *A. thaliana*.

The stable isotope-labeled AQUA peptide can be chemically synthesized in vitro (Brun et al. 2009). On the other hand, all genes encoding several target peptides are concatenated and cloned collectively into one vector and expressed in *E. coli* as an artificial protein (QconCAT protein; Beynon et al. 2005, Kito et al. 2007). Therefore, peptides derived from the artificial QconCAT protein by trypsin digestion should yield a fraction in which tryptic peptides are equally enriched and theoretically have the same stoichiometry. Absolute quantification using concatenated signature peptides encoded by QconCAT genes would be difficult, because there is a possibility of protein contamination from *E. coli* during purification. Nevertheless, the QconCAT method should be utilized more commonly as an internal standard for highly accurate relative quantification, because preparation of the QconCAT protein is much cheaper than synthesizing the AQUA peptide. Quantification methods utilizing internal standards coupled with MS have also been used rather than immunoblotting or enzyme-linked immunosorbent assay (Lehmann et al. 2008).

Label-free relative quantification methods may also be used (Ishihama et al. 2005, Cox and Mann 2008). For example, the dynamin-like protein DRP1A was identified as a PIN1 auxin efflux carrier-interacting protein by AP-MS analysis (Mravec et al. 2011). PIN1-associated proteins have been purified from *A. thaliana* plants carrying the GFP-fused PIN1 and identified by liquid chromatograpy (LC)-MS. DRP1A was identified as one of the most enriched proteins compared with the wild-type plant as a negative control by the label-free relative quantification method using MaxQuant software. Stoichiometry analysis is a promising method for identifying component exchange within protein complexes depending on environmental stress and developmental stages.

## Identification methods for minor proteins

One problem in proteomics is that peptides from abundant proteins are often preferentially detected by MS compared with low-abundant proteins due to ionization suppression. In the case of plants, chloroplast and storage proteins are the major abundant proteins in addition to ribosomal and cytoskeletal proteins (Chen and Harmon 2006). These proteins are often identified in AP-MS analyses as false-positive proteins. In addition, peptides derived from keratins also interfere with peptide detection in MS analyses. Besides improvements in MS, a number of methodologies have been developed to solve this problem. Multidimensional protein identification technology (MudPIT) has been developed and used to separate peptides in high resolution for shotgun analysis (Whitelegge 2002, Kota and Goshe 2011). In MudPIT, a strong cation exchange column and a reverse-phase column are combined for two-dimensional HPLC. This technology has been applied to leaf and flower proteomics (Lee et al. 2007, Feng et al. 2009), as well as ubiqutin proteomics in *A. thaliana* (Maor et al. 2007), although there are no reports showing the use of MudPIT being coupled with a PPI analysis.

Furthermore, Miyamoto et al. (2008) proposed the use of a long monolithic silica-C18 capillary column to separate peptides using a one-shot analysis approach, suggesting that this reduces total MS analysis time and the number of protein samples compared with those in the MudPIT technique. Using a one-shot analysis, 2,602 proteins including 803 membrane proteins were identified in 4 μg of trypsin-digested peptide from *E. coli* (Iwasaki et al. 2010). In contrast, 1,930 proteins were identified using MudPIT analysis by merging 30 LC-MS analysis results from triplicate analyses. More than 42 μg of protein and 18 h more time are needed for the MudPIT analysis to obtain results similar to the one-shot analysis.

The OFFGEL electrophoresis approach, which combines isoelectric focusing using immobilized pH gradient strips with a liquid phase (Hörth et al. 2006, Hubner et al. 2008, Malmström et al. 2009) and filter-aided sample preparation (FASP; Wiśniewski et al. 2009), should be mentioned. The FASP method was developed to increase the recovery rate of protein samples including membrane proteins using SDS, although the MudPIT, the one-shot analysis by long column and the OFFGEL methods were developed for higher resolution peptide separation. SDS interferes with trypsin activity during in-solution digestion, although it is an effective reagent for extracting insoluble proteins such as membrane proteins. In the FASP method, the solubilized sample in 4% SDS is used for in-solution digestion by trypsin after removing SDS and exchanging buffers (Wiśniewski et al. 2009); in that study, the authors identified 7,093 proteins from HeLa cells using this approach, compared with only 3,979 proteins with the OFFGEL method. These advanced methods lead to in-depth proteomics analyses and could allow for the identification of low-abundance proteins via AP-MS analysis.

## Conclusions and future perspectives

Besides the several major approaches for PPI analyses reviewed here, protein array has been developed (Kersten et al. 2003)

and has been used successfully to identify PPIs with calmodulins (Popescu et al. 2007) and protein kinases (Popescu et al. 2009) in *A. thaliana*. Most PPI methodologies have been developed in yeast because of its simplicity, and then applied to several model organisms including *A. thaliana* and rice. However, these methods are sometimes not suitable for plant systems. For example, Ehlert et al. (2006) developed a protoplast two-hybrid system for confirming PPIs in planta. That system was successfully established for large-scale PPI analysis of transcription factors (Wehner et al. 2011).

Development of the plant proteomics field is proceeding at a slow pace. In particular, proteomic approaches are still challenging in non-model plants due to the lack of complete genomic information (Carpentier et al. 2008, Remmerie et al. 2011). Nevertheless, substantial efforts are now in progress to apply the knowledge from model plants to useful plants such as crops and fruits (García-Cañas et al. 2011, Langridge and Fleury 2011, Palma et al. 2011). However, there is still a need to perform proteomics directly on crops and fruits. It is gratifying that genome sequences of many useful plants are being gradually unraveled with the development of next-generation sequencing technology. More recently, the International Plant Proteomics Organization (www.inppo.com), which is a recent global initiative to develop and improve connections between plant proteomics researchers and related fields, has been established (Agrawal et al. 2011b). This cooperative framework will help to develop breakthroughs in the near future.

## Funding

Y.F. was supported by Plant Global Education Project from NAIST, JAPAN, supported by MEXT.

## Acknowledgments

The author appreciates the contribution of Professor Ikuko Hara-Nishimura and Dr. Kentaro Tamura (Kyoto University), where a part of their data is presented in **Fig. 1**. I thank Professor Randeep Rakwal (University of Tsukuba) and Dr. Ali Ferjani (Tokyo Gakugei University) for critically reading the manuscript.

## References

Abe, M., Fujiwara, M., Kurotani, K.I., Yokoi, S. and Shimamoto, K. (2008) Identification of dynamin as an interactor of rice GIGANTEA by tandem affinity purification (TAP). *Plant Cell Physiol.* 49: 420–432.

Agrawal, G.K., Bourguignon, J., Rolland, N., Ephritikhine, G., Ferro, M. et al. (2011a) Plant organelle proteomics: collaborating for optimal cell function. *Mass Spectrom. Rev.* 30: 772–853.

Agrawal, G.K., Job, D., Zivy, M., Agrawal, V.P., Bradshaw, R.A., Dunn, M.J. et al. (2011b) Time to articulate a vision for the future of plant proteomics—a global perspective: an initiative for establishing the International Plant Proteomics Organization (INPPO). *Proteomics* 11: 1559–1568.

Agrawal, G.K. and Rakwal, R. (2008) *In* Plant Proteomics: Technologies, Strategies, and Applications. John Wiley & Sons, Inc., Hoboken, NJ.

Ahrens, C.H., Brunner, E., Qeli, E., Basler, K. and Aebersold, R. (2010) Generating and navigating proteome maps using mass spectrometry. *Nat. Rev. Mol. Cell Biol.* 11: 789–801.

Arabidopsis Genome Initiative. (2000) Analysis of the genome sequence of the flowering plant Arabidopsis thaliana. *Nature* 408: 796–815.

Arabidopsis Interactome Mapping Consortium. (2011) Evidence for network evolution in an Arabidopsis interactome map. *Science* 333: 601–607.

Baginsky, S. (2009) Plant proteomics: concepts, applications, and novel strategies for data interpretation. *Mass Spectrom. Rev.* 28: 93–120.

Batelli, G., Verslues, P.E., Agius, F., Qiu, Q., Fujii, H., Pan, S. et al. (2007) SOS2 promotes salt tolerance in part by interacting with the vacuolar H+-ATPase and upregulating its transport activity. *Mol. Cell Biol.* 27: 7781–7790.

Beynon, R.J., Doherty, M.K., Pratt, J.M. and Gaskell, S.J. (2005) Multiplexed absolute quantification in proteomics using artificial QCAT proteins of concatenated signature peptides. *Nat. Methods* 2: 587–589.

Bindschedler, L.V. and Cramer, R. (2011) Quantitative plant proteomics. *Proteomics* 11: 756–775.

Bonetta, L. (2010) Protein–protein interactions: interactome under construction. *Nature* 468: 851–854.

Boruc, J., Van den Daele, H., Hollunder, J., Rombauts, S., Mylle, E., Hilson, P. et al. (2010) Functional modules in the Arabidopsis core cell cycle binary protein–protein interaction network. *Plant Cell* 22: 1264–1280.

Brandão, M.M., Dantas, L.L. and Silva-Filho, M.C. (2009) AtPIN: Arabidopsis thaliana protein interaction network. *BMC Bioinformatics* 10: 454.

Brandizzi, F., Fricker, M. and Hawes, C. (2002) A greener world: the revolution in plant bioimaging. *Nat. Rev. Mol. Cell Biol.* 3: 520–530.

Breitkreutz, B.J., Stark, C. and Tyers, M. (2003) The GRID: the General Repository for Interaction Datasets. *Genome Biol.* 4: R23.

Brun, V., Masselon, C., Garin, J. and Dupuis, A. (2009) Isotope dilution strategies for absolute quantitative proteomics. *J. Proteomics* 72: 740–749.

Carpentier, S.C., Panis, B., Vertommen, A., Swennen, R., Sergeant, K., Renaut, J. et al. (2008) Proteome analysis of non-model plants: a challenging but powerful approach. *Mass Spectrom. Rev.* 27: 354–377.

Chang, I.F. (2006) Mass spectrometry-based proteomic analysis of the epitope-tag affinity purified protein complexes in eukaryotes. *Proteomics* 6: 6158–6166.

Chen, S. and Harmon, A.C. (2006) Advances in plant proteomics. *Proteomics* 6: 5504–5516.

Cho, Y.H., Yoo, S.D. and Sheen, J. (2006) Regulatory functions of nuclear hexokinase1 complex in glucose signaling. *Cell* 127: 579–589.

Cox, J. and Mann, M. (2008) MaxQuant enables high peptide identification rates, individualized p.p.b.-range mass accuracies and proteome-wide protein quantification. *Nat. Biotechnol.* 26: 1367–1372.

Cox, J. and Mann, M. (2011) Quantitative, high-resolution proteomics for data-driven systems biology. *Annu. Rev. Biochem.* 80: 273–299.

Cristea, I.M., Williams, R., Chait, B.T. and Rout, M.P. (2005) Fluorescent proteins as proteomic probes. *Mol. Cell Proteomics* 4: 1933–1941.

Ehlert, A., Weltmeier, F., Wang, X., Mayer, C.S., Smeekens, S., Vicente-Carbajosa, J. et al. (2006) Two-hybrid protein–protein interaction analysis in Arabidopsis protoplasts: establishment of a heterodimerization map of group C and group S bZIP transcription factors. *Plant J.* 46: 890–900.

Eubel, H., Braun, H.P. and Millar, A.H. (2005) Blue-native PAGE in plants: a tool in analysis of protein–protein interactions. *Plant Methods* 1: 11.

Feng, B., Li, L., Zhou, X., Stanley, B. and Ma, H. (2009) Analysis of the Arabidopsis floral proteome: detection of over 2000 proteins and evidence for posttranslational modifications. *J. Integr. Plant Biol.* 51: 207–223.

Fields, S. and Song, O. (1989) A novel genetic system to detect protein–protein interactions. *Nature* 340: 245–246.

Fukao, Y., Ferjani, A., Fujiwara, M., Nishimori, Y. and Ohtsu, I. (2009) Identification of zinc-responsive proteins in the roots of Arabidopsis thaliana using a highly improved method of two-dimensional electrophoresis. *Plant Cell Physiol.* 50: 2234–2239.

García-Cañas, V., Simó, C., León, C., Ibáñez, E. and Cifuentes, A. (2011) MS-based analytical methodologies to characterize genetically modified crops. *Mass Spectrom. Rev.* 30: 396–416.

Gavin, A.C., Bösche, M., Krause, R., Grandi, P., Marzioch, M., Bauer, A. et al. (2002) Functional organization of the yeast proteome by systematic analysis of protein complexes. *Nature* 415: 141–147.

Geisler-Lee, J., O'Toole, N., Ammar, R., Provart, N.J., Millar, A.H. and Geisler, M. (2007) A predicted interactome for Arabidopsis. *Plant Physiol.* 145: 317–329.

Goff, S.A., Ricke, D., Lan, T.H., Presting, G., Wang, R., Dunn, M. et al. (2002) A draft sequence of the rice genome (Oryza sativa L. ssp. japonica). *Science* 296: 92–100.

Gu, H., Zhu, P., Jiao, Y., Meng, Y. and Chen, M. (2011) PRIN: a predicted rice interactome network. *BMC Bioinformatics* 12: 161.

Haynes, P.A. and Roberts, T.H. (2007) Subcellular shotgun proteomics in plants: looking beyond the usual suspects. *Proteomics* 7: 2963–2975.

Heck, A.J.R. (2008) Native mass spectrometry: a bridge between interactomics and structural biology. *Nat. Methods* 5: 927–933.

Hörth, P., Miller, C.A., Preckel, T. and Wenz, C. (2006) Efficient fractionation and improved protein identification by peptide OFFGEL electrophoresis. *Mol. Cell Proteomics* 5: 1968–1974.

Hubner, N.C., Ren, S. and Mann, M. (2008) Peptide separation with immobilized pI strips is an attractive alternative to in-gel protein digestion for proteome analysis. *Proteomics* 8: 4862–4872.

International Rice Genome Sequencing Project. (2005) The map-based sequence of the rice genome. *Nature* 436: 793–800.

Ishihama, Y., Oda, Y., Tabata, T., Sato, T., Nagasu, T., Rappsilber, J. et al. (2005) Exponentially modified protein abundance index (emPAI) for estimation of absolute protein amount in proteomics by the number of sequenced peptides per protein. *Mol. Cell Proteomics* 4: 1265–1272.

Iwasaki, M., Miwa, S., Ikegami, T., Tomita, M., Tanaka, N. and Ishihama, Y. (2010) One-dimensional capillary liquid chromatographic separation coupled with tandem mass spectrometry unveils the *Escherichia coli* proteome on a microarray scale. *Anal. Chem.* 82: 2616–2620.

Jaillais, Y., Santambrogio, M., Rozier, F., Fobis-Loisy, I., Miège, C. and Gaude, T. (2007) The retromer protein VPS29 links cell polarity and organ initiation in plants. *Cell* 130: 1057–1070.

Jorrín, J.V., Maldonado, A.M. and Castillejo, M.A. (2007) Plant proteome analysis: a 2006 update. *Proteomics* 7: 2947–2962.

Kaake, R.M., Wang, X. and Huang, L. (2010) Profiling of protein interaction networks of protein complexes using affinity purification and quantitative mass spectrometry. *Mol. Cell Proteomics* 9: 1650–1665.

Kang, S.U., Heo, S. and Lubec, G. (2011) Mass spectrometric analysis of GABAA receptor subtypes and phosphorylations from mouse hippocampus. *Proteomics* 11: 2171–2181.

Kato, N., Fujikawa, Y., Fuselier, T., Adamou-Dodo, R., Nishitani, A. and Sato, M.H. (2010) Luminescence detection of SNARE–SNARE interaction in Arabidopsis protoplasts. *Plant Mol. Biol.* 72: 433–444.

Kersten, B., Feilner, T., Kramer, A., Wehrmeyer, S., Possling, A., Witt, I. et al. (2003) Generation of Arabidopsis protein chips for antibody and serum screening. *Plant Mol. Biol.* 52: 999–1010.

Kito, K., Ota, K., Fujita, T. and Ito, T. (2007) A synthetic protein approach toward accurate mass spectrometric quantification of component stoichiometry of multiprotein complexes. *J. Proteome Res.* 6: 792–800.

Klodmann, J., Senkler, M., Rode, C. and Braun, H.P. (2011) Defining the protein complex proteome of plant mitochondria. *Plant Physiol.* 157: 587–598.

Kosová, K., Vítámvás, P., Prášil, I.T. and Renaut, J. (2011) Plant proteome changes under abiotic stress—contribution of proteomics studies to understanding plant stress response. *J. Proteomics* 74: 1301–1322.

Kota, U. and Goshe, M.B. (2011) Advances in qualitative and quantitative plant membrane proteomics. *Phytochemistry* 72: 1040–1060.

Lalonde, S., Ehrhardt, D.W., Loqué, D., Chen, J., Rhee, S.Y. and Frommer, W.B. (2008) Molecular and cellular approaches for the detection of protein–protein interactions: latest techniques and current limitations. *Plant J.* 53: 610–635.

Lalonde, S., Sero, A., Pratelli, R., Pilot, G., Chen, J., Sardi, M.I. et al. (2010) Molecular and cellular approaches for the detection of protein–protein interactions: latest techniques and current limitations. *Plant J.* 53: 610–635.

Langridge, P. and Fleury, D. (2011) Making the most of 'omics' for crop breeding. *Trends Biotechnol.* 29: 33–40.

Lee, J., Garrett, W.M. and Cooper, B. (2007) Shotgun proteomic analysis of Arabidopsis thaliana leaves. *J. Sep. Sci.* 30: 2225–2230.

Lehmann, U., Wienkoop, S., Tschoep, H. and Weckwerth, W. (2008) If the antibody fails—a mass western approach. *Plant J.* 55: 1039–1046.

Li, P., Zang, W., Li, Y., Xu, F., Wang, J. and Shi, T. (2011) AtPID: the overall hierarchical functional protein interaction network interface and analytic platform for *Arabidopsis*. *Nucleic Acids Res.* 39: D1130–D1133.

Li, Y. (2011) The tandem affinity purification technology: an overview. *Biotechnol. Lett.* 33: 1487–1499.

Lilley, K.S. and Dupree, P. (2007) Plant organelle proteomics. *Curr. Opin. Plant Biol.* 10: 594–599.

Lin, M., Hu, B., Chen, L., Sun, P., Fan, Y., Wu, P. et al. (2009) Computational identification of potential molecular interactions in Arabidopsis. *Plant Physiol.* 151: 34–46.

Lin, M., Shen, X. and Chen, X. (2011a) PAIR: the predicted Arabidopsis interactome resource. *Nucleic Acids Res.* 39: D1134–D1140.

Lin, M., Zhou, X., Shen, X., Mao, C. and Chen, X. (2011b) The predicted Arabidopsis interactome resource and network topology-based systems biology analyses. *Plant Cell* 23: 911–922.

Malmström, J., Beck, M., Schmidt, A., Lange, V., Deutsch, E.W. and Aebersold, R. (2009) Proteome-wide cellular protein concentrations of the human pathogen Leptospira interrogans. *Nature* 460: 762–765.

Maor, R., Jones, A., Nühse, T.S., Studholme, D.J., Peck, S.C. and Shirasu, K. (2007) Multidimensional protein identification technology (MudPIT) analysis of ubiquitinated proteins in plants. *Mol. Cell Proteomics* 6: 601–610.

Miernyk, J.A. and Thelen, J.J. (2008) Biochemical approaches for discovering protein–protein interactions. *Plant J.* 53: 597–609.

Millar, A.H., Carrie, C., Pogson, B. and Whelan, J. (2009) Exploring the function–location nexus: using multiple lines of evidence in defining the subcellular location of plant proteins. *Plant Cell* 21: 1625–1631.

Miyamoto, K., Hara, T., Kobayashi, H., Morisaka, H., Tokuda, D., Horie, K. et al. (2008) High-efficiency liquid chromatographic separation utilizing long monolithic silica capillary columns. *Anal. Chem.* 80: 8741–8750.

Mochida, K. and Shinozaki, K. (2011) Advances in omics and bioinformatics tools for systems analyses of plant functions. *Plant Cell Physiol.* 52: 2017–2038.

Monneuse, J.M., Sugano, M., Becue, T., Santoni, V., Hem, S. and Rossignol, M. (2011) Towards the profiling of the Arabidopsis thaliana plasma membrane transportome by targeted proteomics. *Proteomics* 11: 1789–1797.

Moore, I. and Murphy, A. (2009) Validating the location of fluorescent protein fusions in the endomembrane system. *Plant Cell* 21: 1632–1636.

Morsy, M., Gouthu, S., Orchard, S., Thorneycroft, D., Harper, J.F., Mittler, R. et al. (2008) Charting plant interactomes: possibilities and challenges. *Trends Plant Sci.* 13: 183–191.

Mravec, J., Petrášek, J., Li, N., Boeren, S., Karlova, R., Pařezová, M. et al. (2011) Cell plate restricted association of DRP1A and PIN proteins is required for cell polarity establishment in Arabidopsis. *Curr. Biol.* 21: 1055–1060.

Nakagami, H., Sugiyama, N., Ishihama, Y. and Shirasu, K. (2012) Shotguns in the front line: phosphoproteomics in plants. *Plant Cell Physiol.* 53: 118–124.

Okumoto, S. (2010) Imaging approach for monitoring cellular metabolites and ions using genetically encoded biosensors. *Curr. Opin. Biotechnol.* 21: 45–54.

Olinares, P.D.B., Kim, J., Davis, J.I. and van Wijk, K.J. (2011) Subunit stoichiometry, evolution, and functional implications of an asymmetric plant plastid ClpP/R protease complex in arabidopsis. *Plant Cell* 23: 2348–2361.

Palma, J.M., Corpas, F.J. and del Río, L.A. (2011) Proteomics as an approach to the understanding of the molecular physiology of fruit development and ripening. *J. Proteomics* 74: 1230–1243.

Parrish, J.R., Gulyas, K.D. and Finley, R.L. (2006) Yeast two-hybrid contributions to interactome mapping. *Curr. Opin. Biotechnol.* 17: 387–393.

Peng, L., Fukao, Y., Fujiwara, M., Takami, T. and Shikanai, T. (2009) Efficient operation of NAD(P)H dehydrogenase requires supercomplex formation with photosystem I via minor LHCI in Arabidopsis. *Plant Cell* 21: 3623–3640.

Popescu, S.C., Popescu, G.V., Bachan, S., Zhang, Z., Gerstein, M., Snyder, M. et al. (2009) MAPK target networks in Arabidopsis thaliana revealed using functional protein microarrays. *Genes Dev.* 23: 80–92.

Popescu, S.C., Popescu, G.V., Bachan, S., Zhang, Z., Seay, M., Gerstein, M. et al. (2007) Differential binding of calmodulin-related proteins to their targets revealed through high-density Arabidopsis protein microarrays. *Proc. Natl Acad. Sci. USA* 104: 4730–4735.

Qi, Y. and Katagiri, F. (2009) Purification of low-abundance Arabidopsis plasma-membrane protein complexes and identification of candidate components. *Plant J.* 57: 932–944.

Remmerie, N., De Vijlder, T., Laukens, K., Dang, T.H., Lemière, F., Mertens, I. et al. (2011) Next generation functional proteomics in non-model plants: a survey on techniques and applications for the analysis of protein complexes and post-translational modifications. *Phytochemistry* 72: 1192–1218.

Rigaut, G., Shevchenko, A., Rutz, B., Wilm, M., Mann, M. and Séraphin, B. (1999) A generic protein purification method for protein complex characterization and proteome exploration. *Nat. Biotechnol.* 17: 1030–1032.

Rohila, J.S., Chen, M., Cerny, R. and Fromm, M.E. (2004) Improved tandem affinity purification tag and methods for isolation of protein heterocomplexes from plants. *Plant J.* 38: 172–181.

Rohila, J.S., Chen, M., Chen, S., Chen, J., Cerny, R.L., Dardick, C. et al. (2006) Protein–protein interactions of tandem affinity purification-tagged protein kinases in rice. *Plant J.* 46: 1–13.

Rohila, J.S., Chen, M., Chen, S., Chen, J., Cerny, R.L., Dardick, C. et al. (2009) Protein–protein interactions of tandem affinity purified protein kinases from rice. *PLoS ONE* 4: e6685.

Rubio, V., Shen, Y., Saijo, Y., Liu, Y., Gusmaroli, G., Dinesh-Kumar, S.P. et al. (2005) An alternative tandem affinity purification strategy applied to Arabidopsis protein complex isolation. *Plant J.* 41: 767–778.

Sadanandom, A. and Napier, R.M. (2010) Biosensors in plants. *Curr. Opin. Plant Biol.* 13: 736–743.

Schmidt, C., Lenz, C., Grote, M., Lührmann, R. and Urlaub, H. (2010) Determination of protein stoichiometry within protein complexes using absolute quantification and multiple reaction monitoring. *Anal. Chem.* 82: 2784–2796.

Schoonheim, P.J., Veiga, H., da Costa Pereira, D., Friso, G., van Wijk, K.J. et al. (2007) A comprehensive analysis of the 14-3-3 interactome in barley leaves using a complementary proteomics and two-hybrid approach. *Plant Physiol.* 143: 670–683.

Seo, Y.S., Chern, M., Bartley, L.E., Han, M., Jung, K.H., Lee, I. et al. (2011) Towards establishment of a rice stress response interactome. *PLoS Genet.* 7: e1002020.

Sharon, M. and Robinson, C.V. (2007) The role of mass spectrometry in structure elucidation of dynamic protein complexes. *Annu. Rev. Biochem.* 76: 167–193.

Shoemaker, B.A. and Panchenko, A.R. (2007) Deciphering protein–protein interactions. Part I. Experimental techniques and databases. *PLoS Comput. Biol.* 3: e42.

Stark, C., Breitkreutz, B.J., Chatr-aryamontri, A., Boucher, L., Oughtred, R., Livstone, M.S. et al. (2011) The BioGRID Interaction Database: 2011 update. *Nucleic Acids Res.* 39: D698–D704.

Stevens, R.C. (2000) Design of high-throughput methods of protein production for structural biology. *Structure* 8: R177–R185.

Stevenson, S.E., Houston, N.L. and Thelen, J.J. (2010) Evolution of seed allergen quantification—from antibodies to mass spectrometry. *Regul. Toxicol Pharmacol.* 58: S36–S41.

Suter, B., Kittanakom, S. and Stagljar, I. (2008) Two-hybrid technologies in proteomics research. *Curr. Opin. Biotechnol.* 19: 316–323.

Suzuki, Y., Takagi, N., Chimuro, T., Shinohara, A., Sakaguchi, N., Hiratsuka, A. et al. (2011) Design and synthesis of new fluorescent

probe for rapid and highly sensitive detection of proteins via electrophoretic gel stain. *Electrophoresis* 32: 1403–1413.
Swarbreck, D., Wilks, C., Lamesch, P., Berardini, T.Z., Garcia-Hernandez, M., Foerster, H. et al. (2008) The Arabidopsis Information Resource (TAIR): gene structure and function annotation. *Nucleic Acids Res.* 36: D1009–D1014.
Tamura, K., Fukao, Y., Iwamoto, M., Haraguchi, T. and Hara-Nishimura, I. (2010) Identification and characterization of nuclear pore complex components in Arabidopsis thaliana. *Plant Cell* 22: 4084–4097.
Tardif, G., Kane, N.A., Adam, H., Labrie, L., Major, G., Gulick, P. et al. (2007) Interaction network of proteins associated with abiotic stress response and development in wheat. *Plant Mol. Biol.* 63: 703–718.
Templeton, G.W., Nimick, M., Morrice, N., Campbell, D., Goudreault, M., Gingras, A.C. et al. (2011) Identification and characterization of AtI-2, an Arabidopsis homologue of an ancient protein phosphatase 1 (PP1) regulatory subunit. *Biochem. J.* 435: 73–83.
Terpe, K. (2003) Overview of tag protein fusions: from molecular and biochemical fundamentals to commercial systems. *Appl. Microbiol. Biotechnol.* 60: 523–533.
Ueda, M., Manabe, Y. and Mukai, M. (2011) The high performance of 3×FLAG for target purification of a bioactive metabolite: a tag combined with a highly effective linker structure. *Bioorg. Med. Chem. Lett.* 21: 1359–1362.
Uetz, P., Giot, L., Cagney, G., Mansfield, T.A., Judson, R.S., Knight, J.R. et al. (2000) A comprehensive analysis of protein–protein interactions in *Saccharomyces cerevisiae*. *Nature* 403: 623–627.
Uhrig, J.F. (2006) Protein interaction networks in plants. *Planta* 224: 771–781.
Van Aken, O., Pecenková, T., van de Cotte, B., De Rycke, R., Eeckhout, D., Fromm, H. et al. (2007) Mitochondrial type-I prohibitins of Arabidopsis thaliana are required for supporting proficient meristem development. *Plant J.* 52: 850–864.
Van Leene, J., Boruc, J., De Jaeger, G., Russinova, E. and De Veylder, L. (2011) A kaleidoscopic view of the Arabidopsis core cell cycle interactome. *Trends in Plant Sci.* 16: 141–150.
Van Leene, J., Stals, H., Eeckhout, D., Persiau, G., Van De Slijke, E., Van Isterdael, G. et al. (2007) A tandem affinity purification-based technology platform to study the cell cycle interactome in Arabidopsis thaliana. *Mol. Cell Proteomics* 6: 1226–1238.
Van Leene, J., Witters, E., Inzé, D. and De Jaeger, G. (2008) Boosting tandem affinity purification of plant protein complexes. *Trends Plant Sci.* 13: 517–520.
van Wijek, K.J. (2001) Challenges and prospects of plant proteomics. *Plant Physiol.* 126: 501–508.
Vernoux, T., Brunoud, G., Farcot, E., Morin, V., Van den Daele, H., Legrand, J. et al. (2011) The auxin signalling network translates dynamic input into robust patterning at the shoot apex. *Mol. Syst. Biol.* 7: 508.
Warnock, D.E., Fahy, E. and Taylor, S.W. (2004) Identification of protein associations in organelles, using mass spectrometry-based proteomics. *Mass Spectrom. Rev.* 23: 259–280.
Wehner, N., Hartmann, L., Ehlert, A., Böttner, S., Oñate-Sánchez, L. and Dröge-Laser, W. (2011) High-throughput protoplast transactivation (PTA) system for the analysis of Arabidopsis transcription factor function. *Plant J.* 68: 560–569.
Whitelegge, J.P. (2002) Plant proteomics: BLASTing out of a MudPIT. *Proc. Natl Acad. Sci. USA* 99: 11564–11566.
Wienkoop, S. and Weckwerth, W. (2006) Relative and absolute quantitative shotgun proteomics: targeting low-abundance proteins in Arabidopsis thaliana. *J. Exp. Bot.* 57: 1529–1535.
Wiśniewski, J.R., Zougman, A., Nagaraj, N. and Mann, M. (2009) Universal sample preparation method for proteome analysis. *Nat. Methods* 6: 359–362.
Wolters, D.A., Washburn, M.P. and Yates, J.R. 3rd (2001) An automated multidimensional protein identification technology for shotgun proteomics. *Anal. Chem.* 73: 5683–5690.
Xing, D. and Chen, Z. (2006) Effects of mutations and constitutive overexpression of EDS1 and PAD4 on plant resistance to different types of microbial pathogens. *Plant Sci.* 171: 251–262.
Xing, D., Zhao, H. and Li, Q.Q. (2008a) Arabidopsis CLP1-SIMILAR PROTEIN3, an ortholog of human polyadenylation factor CLP1, functions in gametophyte, embryo, and postembryonic development. *Plant Physiol.* 148: 2059–2069.
Xing, D., Zhao, H., Xu, R. and Li, Q.Q. (2008b) Arabidopsis PCFS4, a homologue of yeast polyadenylation factor Pcf11p, regulates FCA alternative processing and promotes flowering time. *Plant J.* 54: 899–910.
Xu, X., Song, Y., Li, Y., Chang, J., Zhang, H. and An, L. (2010) The tandem affinity purification method: an efficient system for protein complex purification and protein interaction identification. *Protein Expr. Purif.* 72: 149–156.
Yu, H., Braun, P., Yildirim, M.A., Lemmens, I., Venkatesan, K., Sahalie, J. et al. (2008) High-quality binary protein interaction map of the yeast interactome network. *Science* 322: 104–110.
Yu, J., Hu, S., Wang, J., Wong, G.K., Li, S., Lin, B. et al. (2002) A draft sequence of the rice genome (Oryza sativa L. ssp. indica). *Science* 296: 79–92.
Zhang, Y., Gao, P. and Yuan, J.S. (2010) Plant protein–protein interaction network and interactome. *Curr. Genomics* 11: 40–46.
Zhao, Q., Brkljacic, J. and Meier, I. (2008) Two distinct interacting classes of nuclear envelope-associated coiled-coil proteins are required for the tissue-specific nuclear envelope targeting of Arabidopsis RanGAP. *Plant Cell* 20: 1639–1651.
Zulak, K.G., Lippert, D.N., Kuzyk, M.A., Domanski, D., Chou, T., Borchers, C.H. et al. (2009) Targeted proteomics using selected reaction monitoring reveals the induction of specific terpene synthases in a multi-level study of methyl jasmonate-treated Norway spruce (Picea abies). *Plant J.* 60: 1015–1030.