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Regulation of developmental processes: insights from mass spectrometry-based proteomics

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Abstract

Mass spectrometry (MS)-based proteomics has become an indispensable tool for protein identification and quantification. In this review, common MS workflows are described, with an emphasis on applications of MS-based proteomics in developmental biology. Progress has been made in the analysis of proteome changes during tissue differentiation and in various genetic perturbations. MS-based proteomics has been particularly useful for identifying novel protein interactions by affinity purification-mass spectrometry (AP-MS), many of which have been subsequently functionally validated and led to the discovery of previously unknown modes of developmental regulation. Quantitative proteomics approaches can be used to study post-translational protein modifications (PTMs) such as phosphorylation, to reveal the dynamics of intracellular signal transduction. Integrative approaches combine quantitative MS-based proteomics with other high-throughput methods, with the promise of a systems level understanding of developmental regulation.

INTRODUCTION

Developmental biology in its modern incarnation grew as an interdisciplinary science at the intersection of embryology, genetics, and molecular biology. It has embraced the advent of “omics” approaches and has greatly benefited from the complete genome sequences of model organisms and an ability to follow the expression of every gene using methods such as global RNA profiling. However, information obtained from genomic experiments is insufficient to fully define cellular functions which are largely carried out by proteins. During organism development, proteins are up- and down-regulated in specific tissues, often without corresponding changes in mRNA abundance¹, post-translationally modified, and engage in interactions with their partners. Proteomics offers a direct way to detect and quantify all of these aspects of protein function, even in complex mixtures. Such experiments can be implemented both on a global scale and in focused, targeted studies. Proteomics has emerged as a powerful new way to analyze how proteins carry out and regulate developmental processes, making it possible to perform experiments that were not feasible before^{2,3}. Proteomic approaches complement other types of large-scale surveys of developmental functions, such as genetic screens and, more recently, transcriptomics, RNAi screens and analysis of chromatin architecture.

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FURTHER READING/RESOURCES

A special issue of the Journal of Proteomics, “Model Organism Proteomics”, vol. 73, issue 11, October 2010. <http://www.sciencedirect.com/science/journal/18743919/73/11>

A special issue of Molecular and Cellular Proteomics, “PROSPECTS: Proteomics in Space and Time”, vol. 11, No. 3, March 2012. <http://www.mcponline.org/content/11/3.toc>

PeptideAtlas: <http://www.peptideatlas.org/>

Recent advances in proteomics have been enabled by the development of instrumentation and analytical software for protein mass spectrometry (MS) ^{2,3}. In this review, common MS-based proteomic workflows are first described, followed by discussion of specific examples of how proteomics has led to fundamental new insights in developmental biology. Emphasis is on the analysis of proteomes in specific tissues and mutant conditions, discovery of novel protein interactions and regulatory functions by affinity purification-mass spectrometry (AP-MS), analysis of post-translational modifications and the dynamics of cell signaling, and integrative approaches that utilize several different techniques for analyzing complex cellular networks. Most examples are drawn from the animal models; readers interested in application of proteomics to plant development are referred to a recent review ⁴.

TECHNOLOGY OF MASS SPECTROMETRY-BASED PROTEOMICS

Shotgun Proteomics

Mass spectrometry relies on the use of instruments that are based on a simple principle: they are able to measure the mass of a biological molecule with exquisite precision. Modern mass spectrometers are equipped with sophisticated components and are achieving ever-increasing resolution and sensitivity, yet the underlying principle of accurate mass measurement remains the same. Current approaches in protein MS can be largely divided into shotgun and targeted proteomics ⁵. Shotgun proteomics (sometimes referred to as discovery, or bottom-up proteomics) is by far the most commonly used method, as it was the first to be developed and is easier to implement. A typical workflow in a shotgun proteomics experiment is outlined in Fig. 1A. Starting with a protein sample, several steps of sample preparation and analysis in a mass spectrometer are carried out with a goal of detecting and identifying as many proteins as possible. Proteins are typically digested with a protease such as trypsin, resulting in a collection of peptides. The peptides are then separated by liquid chromatography (LC), ionized by electrospray ionization (ESI) and sent into the first mass analyzer which detects the mass-to-charge ratios (m/z) of the peptides as they are sequentially eluted during the LC run. This process creates a survey mass spectrum of the sample. In theory, a collection of peptide masses (a “fingerprint”) could unambiguously identify a protein. However, for multi-protein mixtures obtained from complex proteomes this is not feasible, and additional steps of obtaining amino acid sequences of individual peptides are carried out. Peptides are partially fragmented in a collision cell, which creates a random set of shorter peptides that differ from one another by one amino acid. These shorter peptides are then analyzed by a second mass analyzer, which makes it possible to reconstruct the sequence of the peptide based on the known masses of the amino acid side chains (Fig. 1A). The entire procedure is referred to as LC-MS/MS or tandem MS because of the two MS steps.

Since a complex protein sample can generate hundreds and even thousands of peptides, it is difficult to determine the sequence of each peptide entirely from the raw MS/MS data. To aid in peptide identification, bioinformatics analysis of the raw data is performed by comparing the actual spectra with a protein sequence database for the respective organism, digested *in silico*. Clearly, a high-quality proteome annotation is critical for successful identification of proteins in shotgun proteomics experiments. Generally, the greater the number of peptides matched with the database for any given protein, the higher the confidence of this protein's identification. The output from a shotgun proteomics experiment is thus a list of identified proteins that are likely to be present in the starting sample (Fig. 1A). Modern mass spectrometers can now identify hundreds of proteins in complex mixtures in a single LC-MS/MS experiment. The same workflow can be used to search for post-translational protein modifications, because they result in a characteristic shift in mass of the modified peptide by a known amount (Fig. 1D).

Targeted Proteomics: Selected Reaction Monitoring (SRM)

While shotgun proteomics is a good approach to identify proteins in samples whose composition is not known *a priori*, the method relies on a stochastic nature of peptide detection, which is driven by identification of highly abundant proteins. In order to increase reproducibility and sensitivity of detection, targeted proteomics approaches are being developed. In this mode, researchers deliberately focus on a defined set of proteins of interest in the sample, while ignoring all others. Because of the focus on specific peptides, this approach is referred to as selected reaction monitoring (SRM, also known as multiple reaction monitoring or MRM)⁶. SRM methods rely on the use of a modified MS workflow performed on triple quadrupole instruments (Fig. 1B). Sample preparation in terms of trypsin digestion and LC/ESI is similar, but the first mass analyzer is tuned to select only a single specific peptide for a given protein. This peptide is then fragmented in the collision cell, and the second mass analyzer is also set up to measure only a single fragment species from that peptide. The output of such a procedure is referred to as a transition and is a plot of the detected specific fragment intensity over time. Focusing on a single peptide followed by selection of a single product of its fragmentation dramatically increases both the sensitivity and reproducibility of protein detection, making it possible to reliably monitor even low-abundance proteins in complex samples.

SRM assays require prior optimization of the whole procedure, starting from a selection of “good” peptides representing the protein of interest (sometimes referred to as “proteotypic peptides”), and including standardization of every step. Because of a requirement for up-front assay development, SRM applications have been less widespread than shotgun proteomics studies, but their use is likely to broaden as the proteomes are better defined and proteotypic peptide libraries are developed. Large-scale efforts to define detectable proteomes are underway for several model systems, with central repositories such as [PeptideAtlas](#) serving as aggregating databases.

Quantitative Proteomics

The stochastic nature of MS detection makes it impossible to determine the amount of a given peptide without using reference standards. However, it is often desirable to know the exact amount of protein in a given sample, and to be able to compare protein abundances between different samples. Several methods have been developed for both absolute and relative quantification of proteins in MS experiments^{3,7}. Most methods make use of stable isotope labeling of proteins or peptides, though label-free quantification approaches have also been developed. During MS analysis, differentially labeled peptides whose masses may differ by as little as a few daltons can be reliably distinguished, and when compared to each other within a single experiment, provide quantitative information about the relative abundances of each isoform (Fig. 1C).

A variety of labeling approaches are available, which can be broadly divided into *in vivo* (or metabolic) and *in vitro* (chemical) labeling. In metabolic labeling, an organism or a cell line is raised on media containing a precursor labeled with stable (non-radioactive) isotopes which is incorporated into all proteins without affecting their function. In the most popular version of metabolic labeling, termed stable-isotope labeling by amino acids in cell culture (SILAC), an organism or a cell line is grown on media containing lysine or arginine with a precisely defined number of ¹³C and ¹⁵N atoms, making it possible to multiplex the assay⁸. Successful SILAC-based MS experiments are critically dependent on a complete substitution of the natural amino acid with a labeled one, and this has now been achieved for many organisms including yeast, flies, and mice. In the simplest setup, labeled and unlabeled samples from two experimental conditions are analyzed either by a shotgun MS workflow or SRM (Fig. 1A, B). The labeled and unlabeled forms of each peptide are

distinguished by a defined isotopic shift, and their ratios are quantified in a mass spectrometer (Fig. 1C). The result is a list of relative peptide (and hence protein) abundances in two samples.

In chemical, or *in vitro* labeling, protein samples are obtained from two or more experimental conditions, and the proteins or peptides are then chemically labeled with stable isotope-containing reagents. Lately, a procedure employing isobaric tags for relative and absolute quantitation (iTRAQ) and its variations have been gaining in popularity⁹, and simpler (and cheaper) approaches such as dimethyl labeling have also been developed¹⁰. Similar to metabolic labeling, samples are analyzed in a shotgun or SRM workflow, and the relative protein abundances are determined by comparing the intensities of two or more isotopic isoforms derived from the same peptide.

The two main labeling methods (SILAC and *in vitro* approaches) each have advantages and pitfalls. The main advantage of SILAC is the absence of a need for chemical derivatization steps that may add to the experimental error. However, the extent of labeling is critical for a successful experiment and can pose a challenge for certain organisms. While chemical labeling requires more extensive sample processing than SILAC and is more costly, it allows for higher multiplexing and can be applied to samples not amenable to metabolic labeling⁹.

APPLICATIONS OF MASS SPECTROMETRY-BASED PROTEOMICS IN DEVELOPMENTAL BIOLOGY

Profiling Developmental Changes Using Proteomics

What possibilities are offered by modern protein MS to developmental biologists? Despite certain challenges pertaining to the inherent complexity of the proteome (Box 1), MS-based proteomics approaches have been successfully applied in several areas of developmental biology. One of the basic aspects of development is the process of cell and tissue differentiation, and many MS-based proteomics studies have been directed at analyzing the differences in protein composition in various organs and tissues. In *Drosophila*, tissue-specific proteomes were studied in embryos and adult heads, male reproductive system, sperm, wing imaginal discs, larval hemolymph, and neuropeptides (reviewed in¹³). A similar comparative analysis has been carried out in a study of adult zebrafish organs¹⁴. Recent advances in instrumentation and sample preparation are yielding an ever-increasing coverage of the tissue-specific proteomes, e.g. the cardiac proteome in the mouse¹⁵ and *Drosophila*¹⁶. Many tissue profiling experiments have been aided by separation of proteins on two-dimensional gels prior to MS. In a recent example, differences between the ectoderm and mesoderm in early *Xenopus* embryos were analyzed using two-dimensional difference gel electrophoresis (DiGE) followed by MS identification of the differentially expressed proteins¹⁷. In a creative application of differential isotope labeling to study tissue-specific proteins, female flies metabolically labeled with ¹⁵N were mated with unlabeled males, and the male seminal fluid transferred at mating was then analyzed in dissected female reproductive tracts, allowing an unambiguous differentiation between the male and female proteins^{18, 19}. This type of analysis would not be possible without the use of proteomics.

Proteomics is also being used to map changes in protein expression during development as a function of time, in an ever-increasing number of studies in different model systems. For example, maternal-to-zygotic transition (MZT) was studied in *Drosophila* using ¹⁵N metabolically labeled embryos²⁰. This study uncovered widespread post-transcriptional downregulation of maternally expressed proteins during MZT. ¹⁵N metabolic labeling was also recently applied to map proteome changes in *C. elegans* ontogenesis²¹. In the mouse, the proteomes of oocytes at different stages were compared²², and in another example,

proteomics was used to profile postnatal intestinal development²³. In two interesting studies, MS-based proteomics revealed that caste differentiation in bees and wasps is detectable at the protein level earlier than previously thought^{24,25}. A study on gastropod embryo development is noteworthy because the authors had to use *de novo* peptide and protein identification as the genome sequence of their model organism was unavailable²⁶.

In tune with the growth of interest in stem cell biology, MS-based proteomics has been applied to study the differentiation of stem cells. Starting with the first proteomic profiling of human embryonic stem cells²⁷, proteomics has been employed to study many aspects of stem cell biology¹¹. For example, many novel cell surface markers of mouse embryo-derived stem cell lines were identified using MS²⁸. These findings guided validation of antibodies that were subsequently used for isolation of viable lineage progenitors from blastocyst stage embryos by flow cytometry²⁸.

Using Genetics in Combination with Proteomics to Analyze Development

Genetic approaches have contributed tremendously to our understanding of developmental pathways and regulatory mechanisms. MS-based proteomics opens up new possibilities to analyze changes in cellular proteomes using mutants and other genetic perturbations. One of the early efforts compared genetically ventralized and lateralized *Drosophila* embryos and identified proteins involved in ventral furrow morphogenesis during gastrulation²⁹.

Metabolic ¹⁵N labeling was used to discover proteins with altered abundance in the *daf-2* mutant of *C. elegans*, shedding light on *dauer* formation, insulin signaling, and the aging process³⁰. SILAC-labeled worms were recently used to identify proteins that are up- or down-regulated in nuclear hormone receptor 49 (*nhr-49*) mutants and RNAi knockdown animals, revealing downregulation of enzymes related to lipid metabolism³¹. In a pioneering application of SILAC to label a whole mammalian organism, proteome changes were studied in kindlin-3 (*Fermt3*) mutant mice, which uncovered a network of proteins required for normal erythrocyte development³². Analysis of protein abundance in germline-lacking *tudor* mutants of *Drosophila* in combination with SILAC labeling of flies made it possible to discriminate whether sex-specific proteins originated in the germline or somatic tissue³³.

One recently developed application of MS-based proteomics is identification of microRNA (miRNA) targets, which is of interest for developmental biology, given widespread gene regulation by microRNAs. Proteomics is particularly suitable for identifying targets of miRNA regulation, because much of this regulation occurs at the posttranscriptional level. Several studies performed miRNA overexpression or knockdown in cultured cells followed by a SILAC-based quantification of protein abundance, which revealed a widespread effect on protein translation as a consequence of perturbing even a single miRNA^{34–36}.

Discovery of Novel Protein Interactions by Affinity Purification-Mass Spectrometry (AP-MS)

Many developmental regulatory mechanisms involve modulation of a protein's activity through its binding to other proteins. MS-based proteomics has been particularly successful at analyzing protein-protein interactions (PPIs) important in development. Binary PPIs have been studied by yeast two hybrid methods, however MS-based proteomics can be applied to the analysis of complexes formed in living cells, thus better approximating the cellular environment in which proteins interact. Over the years, a method of choice for analyzing PPIs has been affinity purification-mass spectrometry (AP-MS)³⁷. A typical AP-MS workflow is shown in Fig. 2. A protein of interest is tagged with an affinity tag, expressed in cultured cells or in the organism, then a protein complex containing the bait and associated subunits is purified using one or two affinity steps, followed by analysis of the components

with LC-MS/MS. A variety of tags have been used in this workflow, with tandem affinity purification (TAP) tags used for both large-scale and focused projects (reviewed in ^{13, 37}). More recently, single-step purification approaches employing streptavidin binding peptide (SBP) and other tags have been shown to be as effective as TAP ^{38, 39}. The advantages of single-step purification are a significant reduction in time and the potential to identify transiently or weakly associated components.

The output from an AP-MS experiment is a list of putative interacting partners of the protein used as bait (Fig. 2). The identities of the interactors can provide insight into the function of the bait protein through the “guilt by association” principle. In that respect, AP-MS experiments can be viewed as being complementary to genetics screens. Both types of approaches can be used to map and characterize a particular cellular pathway, albeit from somewhat different angles. While genetic screening interrogates gene function and does not assume a direct interaction between components, AP-MS experiments identify an immediate protein interaction network of a protein of interest, without necessarily assigning functional significance to the interactions. PPI data can enrich genetics by providing a molecular framework for interpreting the results of genetic screens, and can even extend the reach of genetics by revealing components that may be missed in a genetic screen. However, because of the functionally unbiased nature of the AP-MS experiments and a potential for identifying false positives, the list of interactors should be considered to be putative until interactions are validated in independent assays and followed up with functional studies.

Several novel protein interactions identified by AP-MS and functionally validated have led to the discovery of new developmental regulatory mechanisms. Some examples of these studies are listed in Table 1. These examples clearly demonstrate that AP-MS has become a powerful technique to advance our knowledge of developmental regulation in different organisms. AP-MS analysis of eukaryotic protein complexes on a large scale has until recently been only carried out in yeast ³⁷. This gap has been filled by a study in *Drosophila* which determined the largest protein interaction map generated by an AP-MS approach for a metazoan ⁵⁰. Though this and many other AP-MS studies employed cell culture as an accessible experimental system and/or mild overexpression, it is clear that many of the interactions identified this way are biologically meaningful (Table 1). However, arguably the best approach to isolate protein complexes is to use transgenic organisms in which the endogenous gene is replaced with a tagged isoform, as has been done in a number of studies ^{45, 47, 51}.

Exciting new developments of the AP-MS technique are starting to be implemented. Various mutations can be introduced into the bait protein and assessed for their effect on complex composition, and changes in protein interactions can also be studied in mutant organisms ². It is now possible to use quantitative proteomics to analyze changes in complex composition during development, as was done using iTRAQ labeling to study Cyclin E-associated proteins in adult vs. embryo mouse brain ⁵². Transient or unstable interactions can be revealed by using *in vivo* cross-linking strategies ^{53, 54}. As only a small fraction of the total PPI space is currently known, AP-MS will continue being a useful tool for identifying new protein interactions and modes of regulation in development.

Analysis of Post-Translational Modifications (PTMs) and Cell Signaling

In addition to PPIs, a common regulatory mechanism to modify protein activity during development is post-translational modifications (PTMs). The most widespread regulatory PTM is phosphorylation, however many other kinds of PTMs are known to be developmentally important, including acetylation, ubiquitination, sumoylation, and glycosylation. MS-based proteomics is extremely well suited to study PTMs because a modified peptide is visible in a mass spectrum as a peak shifted by a characteristic mass

value (see Fig. 1D). For an in-depth review of the technology and applications of phosphoproteomics, see a review by M. Jünger in this series.

In one of the earliest comprehensive studies of PTMs by MS, a quantitative temporal analysis of EGFR phosphorylation was carried out by Mann and colleagues using SILAC labeled HeLa cells⁵⁵. This study revealed thousands of protein phosphorylation events during the cellular response to EGFR stimulation and showed that phosphorylated proteins can be grouped into clusters with different temporal profiles. Quantification of signaling dynamics was taken to a new level in a recent study in which AP-MS was used in combination with SRM (in the so-called AP-SRM method) to follow quantitative changes in protein complex composition and phosphorylation events involving signaling adaptor GRB2, after stimulation of HEK293T cells with growth factors⁵⁶. This analysis revealed both the core interactors as well as growth-factor stimulated GRB2 associations, and also assigned interactions to specific domains with the use of mutated GRB2 isoforms.

Few studies have directly analyzed PTMs in development. A comprehensive survey of protein phosphorylation landscape in the *Drosophila* embryos identified 13,720 different phosphorylation sites in 2,702 proteins⁵⁷. In a recent study, phosphorylation events were analyzed during egg to larva transition in a polychaete⁵⁸. Evidence for significant changes in the phosphoproteome during development was obtained by comparing phosphorylation sites in neonatal vs. adult brain in the mouse⁵⁹.

In addition to phosphorylation, other kinds of developmentally important PTMs are starting to be investigated with MS-based proteomics. For example, glycosylation of the Notch receptor, which is central to the regulation of Notch signaling, was studied with a focus on the sites and dynamics of glycosylation events^{60–62}. Analysis of sumoylation in the early *Drosophila* embryos has identified multiple targets and implicated sumoylation of Ras as an important developmental regulatory mechanism⁶³.

Integrative Approaches

A broader utility of MS-based proteomics as an indispensable part of systems biology approaches is evident from emerging integrative studies that combine proteomics with other high-throughput methods, such as transcriptomics. In one example, a chromatin remodeling regulator ISWI was knocked down by RNAi in *Drosophila* SL2 cells, and the resulting global changes in the transcriptome and the proteome were quantified⁶⁴. This study revealed a widespread non-correlation between changes in protein and transcript levels, suggesting a significant extent of post-transcriptional control of protein abundance in response to perturbation of a major chromatin component. In another example of an integrative approach, data from systematic RNAi screens in *Drosophila* cultured cells were combined with the phosphosite data from the study mentioned above⁵⁷ to construct a JNK (basket) regulatory network⁶⁵. This study provided a systems view of JNK signaling, highlighting functional modules and their interconnections via specific protein phosphorylation sites⁶⁵. A similar approach which combined RNAi screens with AP-MS analysis of protein complexes has comprehensively characterized the RTK-Ras-ERK signaling network and revealed several previously unknown regulators⁶⁶. One of the most comprehensive recent studies tracked a human individual for 14 months with detailed genomic, transcriptomic, metabolomic, and proteomic analyses and revealed changes that correlated with healthy and diseased states occurring during that time period⁶⁷.

CONCLUSION

It is not an exaggeration to say that MS-based proteomics has established itself as an integral component of systems biology approaches, contributing to an emerging discipline of

“systems developmental biology”⁶⁸. We are now in a wave of a “third generation proteomics” that strives to achieve a multidimensional view of proteomes by analyzing protein localization, turnover, and isoforms, in addition to absolute and relative quantification and the study of PPIs and PTMs⁶⁹. Some of the emerging technologies in biological MS include the development of top-down proteomics (analysis of intact proteins) as opposed to bottom-up (shotgun) methods, with the obvious advantage of obviating the need for extra sample preparation steps such as proteolytic digestion. As proteotypic peptide libraries become more widespread, the difference between the shotgun and targeted (SRM) methods may become less distinct⁵. Many analyses are shifting towards protein quantification, in addition to simple identification. While issues related to proteome complexity (Box 1) remain a challenge, there is little doubt that advances in MS-based proteomics technologies will lead to new and unique insights into the regulation of developmental processes.

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BOX 1: Challenges of Working With Complex Tissues

Three important challenges have been encountered when MS-based proteomics methods were applied to study proteins from cells and organisms. The first one stems from an enormous dynamic range in protein abundance that exists in cells, from just a few molecules to thousands of copies per cell. The second relates to the compositional complexity of samples obtained even from a single type of cells. Thus, an average mammalian cell can express over 10,000 polypeptides that can differ in abundance over several orders of magnitude ^{1, 3}. These challenges of protein heterogeneity pose a problem for shotgun proteomics, with its tendency to detect more abundant proteins. To overcome these issues, various methods of sample fractionation and enrichment have been developed, ranging from traditional one- and two-dimensional gel electrophoresis to sophisticated affinity-based methods that enrich for a class of proteins or modifications of interest ^{3, 7, 11}. In addition, better peptide chromatography procedures help tackle proteome complexity. A third challenge in working with complex samples is caused by the limitations in MS sensitivity and a requirement for a significant amount of input material for reliable protein identification (i.e. on the order of 10^6 cells). However, recent instrument improvements have pushed the sensitivity of mass spectrometers to a new level, making it possible to identify proteins from small cell populations, such as those obtainable by FACS ¹¹. Improved resolution of modern MS approaches allows a near-complete identification of all proteins from simpler organisms such as yeast ¹², and it will soon be possible to identify almost all expressed proteins in cells from higher eukaryotes in a single experiment ³.

A. Shotgun proteomics

B. Targeted proteomics: selected reaction monitoring (SRM)

C. Quantitative proteomics

D. Analysis of PTMs

Figure 1.

Common mass spectrometry-based proteomics workflows. (A) Shotgun proteomics. The goal of shotgun proteomics experiments is to discover and identify as many proteins and their post-translational modifications (PTMs) as possible. An example fragmentation process in the collision cell is shown for a random peptide; this process is repeated for all detected peptides. (B) Targeted proteomics: selected reaction monitoring (SRM). The aim of targeted proteomics studies is to quantify a defined, limited set of proteins with high reproducibility and sensitivity. A single precursor peptide and its single fragment chosen for SRM are highlighted in red throughout the procedure. (C) Quantitative proteomics. The goal is to quantify differences in protein abundance between two or more samples, which is achieved by differential labeling of proteins or peptides with stable isotopes. This approach can be used both with shotgun and targeted proteomics methods. (D) Analysis of PTMs. Identification of PTMs is possible due to a mass shift that is characteristic for a particular modification. In the example shown, phosphorylation shifts the mass of a peptide by 80 Da. Abbreviations: LC, liquid chromatography; ESI, electrospray ionization. See text for more details.

Figure 2.

Affinity purification-mass spectrometry (AP-MS) workflow. A protein of interest is tagged with an affinity tag and expressed in cultured cells or *in vivo*. The bait protein and associated subunits are purified using one or more affinity steps, followed by LC-MS/MS identification of components. Novel associations are validated in functional studies.

Table 1

Novel protein associations and corresponding developmental regulatory mechanisms identified by affinity purification-mass spectrometry (AP-MS).

Organism	Bait	Interactor(s)	Tag	Source cell type	Validated function	References
Worm	MIS-12, KBP-1	10 kinetochore proteins	LAP (GFP-S)	Transgenic worms	Outer kinetochore assembly, chromosome segregation	40
Worm	DCR-1	Several associated proteins	8xHA	Transgenic worms	Role of DCR-1 in multiple small-RNA-mediated pathways	41
Fly	Deltex (Dx)	Kurtz (Krtz)	TAP (ProtA-CBP)	Stable Kc167 cell line	Downregulation of Notch signaling	42
Fly	Archipelago (Ago/Fbxw7)	Myc (dm)	TAP (ProtA-CBP)	Stable Kc167 cell line	Degradation of Myc, control of cell growth	43
Fly	Myopic (Mop/PTPN23)	Yorkie (Yki)	GS-TAP (ProtG-SBP)	Stable S2 cell line	Endocytic regulation of Yorkie/Hippo signaling	38
Zebrafish	Elipsa	Intraflagellartransport (IFT) particle proteins	TAP (ProtA-CBP)	Transfected zebrafish embryos	Regulation of intraflagellartransport and ciliogenesis	44
Mouse	Cyclin D1	Transcription factors	FLAG-HA	Mouse organs from tagged knock-in lines	Transcriptional function of Cyclin D1 in development	45
Mouse	Oct4 (POU5F1)	Several associated proteins	3xFLAG and 3xFLAG-CBP	Embryonic stem cell lines	Expanded view of Oct4 regulatory network and pluripotent cell identity	46, 47
Human	Disheveled (Dvl)	KLHL12, Cullin-3 (CUL3)	SBP-HA-CBP	Stable HEK293T cell line	Degradation of Dsh, downregulation of Wnt signaling	48
Human	WTX (FAM123B)	-catenin, other proteins	SBP-HA-CBP	Stable HEK293T cell line	Degradation of -catenin, downregulation of Wnt signaling	49