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REVIEW

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Global views of proteasome-mediated degradation by mass spectrometry

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ABSTRACT

Introduction: Degradation of proteins by cellular proteasomes is critical for the fidelity of protein homeostasis and proper cell function. Indeed, perturbations in proteasome function, as well as the degradation of specific substrates, are associated with a variety of human diseases. Yet, monitoring and analyzing protein degradation in a high throughput manner in physiology and pathology remains limited.

Areas covered: Here we discuss several of the recently developed mass spectrometry-based methods for studying proteasome-mediated cellular degradation and discuss their advantages and limitations. We highlight Mass Spectrometry Analysis of Proteolytic Peptides (MAPP), a method designed to purify and identify proteasome-cleaved cellular proteins as a novel approach in molecular and clinical profiling of human disease.

Expert opinion: The recent improvement of proteomics technologies now offers an unprecedented ability to study disease in clinical settings. Expanding clinical studies to include the degradation landscape will provide a new resolution to complement the cellular proteome. In turn, this holds promise to provide both new disease targets and novel peptide biomarkers which will further enhance personalized proteomics.

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1. The cellular role of degradation

A tight balance between the synthesis and degradation rates of a protein critically regulates its cellular abundance. Targeting defective, dispensable, or obsolete proteins to degradation is an important mechanism required to maintain protein homeostasis, or proteostasis [1]. As degradation of a protein provides a powerful mechanism to shut down a specific cellular function or activity, this level of regulation is crucial for understanding cellular protein dynamics and the principles governing proteasome-mediated control of cellular function. Despite the pivotal role that protein degradation plays in regulating the cellular environment, our ability to analyze changes in degradation, in a proteome-wide manner, remains limited. Here, we discuss recent advances in mass spectrometry (MS)-based methods for the detection of protein degradation and highlight the advantages and limitations of our recently developed method of proteasome footprinting. Detection of lysosomal proteolysis and non-MS-based methods for analyzing changes in protein degradation are covered elsewhere [2,3].

The stability of numerous proteins involved in diverse cellular functions, such as the cell cycle or response to inflammatory signals, is regulated by the extensive network of the ubiquitin-proteasome system. Among the most investigated examples are p53 and IkB, whose stability is controlled to allow cells to modulate their response to apoptotic and inflammatory cues, respectively. Indeed the tight regulation of their degradation is critical for ensuing rapid cellular responses [4–6]. For most of the proteins characterized to date, their cellular function was studied prior to their stability or mode of degradation. Notwithstanding the importance of identifying alterations in the degradation of specific proteins under various conditions, examining the degradation landscape can also help uncover potential links between proteasome function, proteolysis, and human disease. Perturbations in proteasome function have already been implicated in neurodegeneration, cancer, cardiac dysfunction, viral infection and other pathologies [7,8]. Aging was also linked with a decrease in the clearance of misfolded proteins and ultimately the buildup of harmful protein aggregates [8]. Furthermore, recent studies have associated alterations in proteasome structure and function to the response to oxidative stress, a hallmark of aging [9]. Thus, analyzing the cellular 'degradome' in various tissues and cell types across different pathologies may offer insight into both protein- and proteasome-specific aberrations in proteolysis. Yet, this level of regulation, namely proteasomal degradation, has been largely missing thus far in both molecular and clinical profiling.

2. Assessing protein turnover

It has been known for several decades that there are proteins that are degraded by the proteasome at a rate dependent on the age of the protein, where recently translated proteins were shown to have a faster turnover rate than long-lived ones [10]. However, whether these are underlying principles and whether they hold true for different classes of proteins remained unclear as these studies were done in bulk on

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specific proteins and could not provide the necessary resolution for proteome-wide analysis. Improvements in MS analysis have provided tremendous capabilities in measurements of protein half-life by improving the depth and sensitivity of analyzing complex biological samples. For example, quantitative analyses that employ metabolic labeling of proteins using stable isotope labeling by amino acids in cell culture (SILAC) allowed for better quantification of protein abundances than MS without labeling [11]. A combination of SILAC with pulse-labeling following proteasome inhibition identified the specific rates of degradation for thousands of cellular proteins [12]. One observation emerging from this study is that many of the proteins with age-dependent degradation were found to be subunits of cellular protein complexes, positing that the incorporation of a protein into a complex may stabilize it. Thus, the ubiquitin-proteasome system is involved in balancing the cellular environment and removing non-stoichiometric subunits from the cell.

One major limitation in examining proteasome-dependent degradation in studies relying on labeled MS analysis is the requirement of inhibiting the proteasome genetically or pharmacologically. Since proteasome inhibition induces cellular stress, it is unclear whether proteins that increase in abundance following inhibition are those that were destined for degradation or those whose expression was induced by the stress (i.e. proteasome inhibition). Thus, there is a need for a direct method of studying proteasome-mediated degradation without proteasome inhibition to decouple between the effects of inhibition on the cell and the normal cellular degradation program.

3. Proteome-wide identification of ubiquitinated proteins

Another MS-based method, which was developed to identify proteins targeted for degradation, involved isolating and identifying ubiquitinated proteins following immunoprecipitation with antibodies against different ubiquitin moieties or the terminal glycine residues of ubiquitin following tryptic digest [13,14]. Reversing the order of the protocol and digesting the sample with trypsin and only then isolating remnants of ubiquitin modifications with the diGly antibody, coupled with advances in MS, led to the identification of hundreds of ubiquitinated proteins in HEK293 cells [14]. This system has been widely employed to identify ubiquitinated proteins in various systems. However, as not all ubiquitination events target proteins for degradation and may also be involved in signaling events, this system was combined with proteasome inhibition to identify ubiquitinated targets that accumulated upon inhibition [15,16]. Recently K48-linked ubiquitin antibodies and Tandem Ubiquitin Binding Entities (TUBEs) against specific ubiquitin chains have been developed, contributing to the arsenal of available tools for detecting substrates that are destined for proteasomal degradation [16,17].

4. Degradation by cellular proteasomes

While isolating ubiquitinated proteins enriches for the population of proteins sent for proteasome degradation, the most direct way to determine if a protein is degraded by the proteasome is to capture the protein fragments following proteasomal cleavage. Peptide cleavage occurs in the catalytic core of the proteasome, comprised of 28 subunits arranged as a barrel with 4 stacked rings (two α and 2 β rings). Three of these subunits are responsible for cleaving entering polypeptides [18]. Seminal studies used in vitro purified proteasomes fed with model substrates to reveal the protease cleavage motifs of the catalytic subunits of the proteasome, identifying the caspase-, trypsin-, and chymotrypsin-like proteasome cleavages of the β1, β2, and β5 subunits, respectively. In vitro studies were also used to characterize the cleavage properties of a different type of proteasomes called immunoproteasomes. Immunoproteasome are basally expressed mainly in immune cells and upon inflammatory signaling they are upregulated in additional cell types, such as epithelial cells. Immunoproteasomes have an additional chymotryptic-like subunit, which cleaves following hydrophobic amino acids, instead of the caspase-like in the constitutive proteasome. This change in proteasome species is thought to generate peptides that are biochemically favorable for antigen loading onto MHC, thereby altering antigen presentation [19]. Indeed, global analysis of synthetic peptides verified differences in the cleavage motif produced by different types of proteasomes in vitro [20].

Aside from the catalytic core, proteasomes contain regulatory caps such as the 19S or 11S complexes, which are responsible for substrate recognition, removal of ubiquitin and substrate unfolding. The 19S regulatory cap may also be regulated by cellular signals and cellular localization to affect proteasome composition and function. Thus, examining flux through the proteasome, in cellular systems, may provide novel insight into regulatory principles of cellular proteolysis which cannot be extracted merely by identifying the proteins that are targeted for degradation. Collectively, the effects of different regulatory components, cleavage motifs, the selectivity of proteasome targets and novel sequence determinants important for proteasomal degradation may be discovered by examining cleaved proteasomal peptides directly.

5. Cellular proteasomal footprinting

In an attempt to address some of these challenges, we have recently developed an approach of mass spectrometry analysis of proteolytic peptides (MAPP), which captures peptides that were cleaved by cellular proteasomes [21]. In short, cellular lysates are cross-linked prior to affinity purification of cellular proteasomes. To separate proteasomes and other full-length proteins from peptides, reverse-phase chromatography is performed. Proteasome-cleaved peptides or peptides that are associated to the proteasomes are then eluted and sent for identification by MS. Thus, MAPP offers the ability to directly analyze naturally-cleaved peptides from cellular proteins targeted to degradation. As MAPP detects peptides that were recently cleaved, reflects a snapshot of cellular proteolysis. Indeed, our analysis showed that on average, proteins with higher turnover were more readily detected by MAPP than in standard bottom-up proteomics. Further, in studying cells stimulated with inflammatory cytokines, while only 6% of the

detected proteome changed in abundance following stimulation, 24% of the MAPP-detected degradome was altered by the cytokine stimulation [21]. This suggests that the degradome reflects an actively regulated portion of the general cellular proteome and therefore MAPP may detect events that may be missed by bottom-up proteomics.

Importantly, MAPP may provide the ability to identify proteins that are degraded in a specific cellular compartment (e.g. nucleus) as long as proteasomes from that compartment were retained in the lysed material. For example, two copies of a protein with an identical amino acid sequence, and identical identification in MS may have different stability depending on their cellular localization, interacting partners of the protein (e.g. complex), and its protein modifications [12,22]. One such case was observed in comparing clinical samples of peripheral blood mononuclear cells (PBMCs) from patients with Systemic Lupus Erythematosus (SLE) or and healthy individuals. While changes in protein degradation by MAPP were sufficient to distinguish the diseased and healthy individuals, the analysis of bulk protein populations by bottom-up proteomics of the same samples could not. Specifically, we found that histone degradation was altered between the healthy and SLE PBMCs [21]. As histones are involved in the pathogenesis of SLE, our results suggest a role for aberrant proteasomal degradation in SLE manifestation [23]. These results raise the intriguing possibility that anomalies in histone degradation and other cellular proteins may potentially generate autoantigens and induce self-antigenicity and immune response. Deciphering the molecular mechanisms that underlie the differential histone degradation (e.g. specific post-translational modifications), as well as identification of the specific cell types that are responsible for this phenomenon, may be an important direction for investigation. Nevertheless, a clear advantage of direct detection of degradation products using MAPP comes from the ability to extract meaningful information from clinical samples in which the biological sample is usually limited.

It will be intriguing to examine the utilization of MAPP in clinical proteomics for detecting alterations in proteolytic activities due to specific mutations, changes in expression or perturbations in protein homeostasis in human diseases such as autoimmunity, neurodegeneration and cancer. The utilization of proteasome inhibitors in the clinic as the first line of treatment in malignancies such as multiple myeloma and mantle B cell lymphoma highlights the need to examine direct changes in the proteolytic activity of constitutive and immunoproteasomes. The ability to assess the effect of different inhibitors on the degradation landscape both in vitro and in vivo should afford new ways to evaluate their mechanism of action and downstream effect on the cellular environment. Thus, introducing profiling of the degradation landscape of different proteasome populations, from different cell types and clinical samples and from a wide range of pathologies may uncover biological processes and cellular regulation which are currently missed.

6. Characterizing proteasome-cleaved peptides

Another important feature of MAPP is that it retains the information of naturally-cleaved peptides. This is a key difference in the processing of samples between MAPP and bottom-up proteomics, as in the latter proteins are cleaved into peptides by an exogenous enzyme (e.g. Trypsin) which alters the cleavage patterns of cellular peptides. By aligning MAPP-identified peptides onto the full-length amino acid sequence of the parent protein we are faced with several observations that require further exploration. Some regions have a lower protein coverage; these may be due to shorter peptides that were not detected by MS or may reflect biologically relevant information (e.g. peptides that are loaded onto MHC). Alternately, changes in how peptides bind to proteasomes, are retained by the complex, or the speed of their cleavage and diffusion may also influence the specific peptides detected from a given protein [18,24,25]. As peptides cleaved by the proteasome serve as the source of MHC class I-presented peptides, comparing epitopes identified by MAPP and immunopeptidomics analyses may yield insight into determinants of peptide selection and presentation. Thus, it is still early to know the full extent of information we will gain by analyzing the specific peptides identified by proteasome footprinting.

7. Future challenges and directions

MAPP specifically, and the fields of proteomics and peptidomics in general, have limitations that will need to be addressed in the coming years of method developments. Because MAPP relies on MS for protein identification, peptides that are shorter than 6 amino acids or longer than 40 will not be identified as this reaches the identification limit of the MS. In addition, because the method makes use of label-free quantification, protein intensities are only relative and do not reflect the precise quantity of peptides present. There are also technical limitations to the method. While we utilized cross-linking to enable peptide isolation, further optimization of the system should allow detailed examination of the precise effects the cross-linking step may have on proteasome function and cleavage. Even with such data at hand, there is a remaining challenge in the analysis of non-tryptic peptides, as the field of peptidomics is still in its infancy as compared to the broader proteomics field [26]. Most MS analysis tools were designed primarily for tryptic digest, where every peptide ends in either an arginine or lysine. However, endogenous cleavage, especially by a combination of proteases such as the proteasome, results in a greater variety of peptide start and end sites and adds great complexity to the bioinformatics analyses and protein inference. Instead of matching MS/MS spectra to the set of peptides cleaved at a confined set of residues, search spaces now need to contain peptides of a wide variety of lengths and with every potential terminal residue. With a greater variety of potential peptide products, downstream analysis such as protein abundance inference or post-translational modifications will require new methods of spectrum interpretation that can combat search space expansion.

In addition to the computational challenges, there are biological nuances to consider when interpreting data from MAPP experiments. Direct comparison of K48 ubiquitinated species [16,17] and MAPP-identified targets may be utilized for validation of degraded substrates and for greater

understanding of their dynamics of degradation. However, for both standard proteomics and MAPP-identified peptides, an increase in peptide/protein intensity under a given condition can reflect an increase in the abundance of the protein, in correlation with an increase in degradation products. Alternately, an increase in protein degradation may reflect a decrease in protein abundance in the cell, as more copies are being degraded. As MAPP and K48-ubiquitinated species identify proteins at different stages in the degradation process, they expected to provide complimentary yet differing views of degradation as they sample substrates before and after proteasome cleavage, respectively.

Even with these current limitations, the ability to probe the active degradation landscape with MAPP should highlight novel aspects of the dynamic regulation of the proteome. One can ponder on additional utilities by drawing parallels from the investigation of active translation by the introduction of Ribo-seq [27]. Both methods, Ribo-seq and MAPP, offer a glimpse into actively regulated processes that the static snapshots of classical transcriptomics and proteomics could not provide (Figure 1). Ribosome footprinting identifies actively translated sequences which are protected by the ribosome, whereas proteasome footprinting analyzes peptide fragments of proteasome-degraded proteins that were captured within or adjacent to proteasomes. Upon the advent of Ribo-seq, new insight into the regulation of translation by the ribosome, the complexity of cellular mRNA regulation, mRNA dynamics, and temporal and localized regulation was revealed. Although proteomic analyses are still lagging behind with their throughput and quantitative abilities, we expect that further development of MAPP and comparable MS-based methods can shed new light on active cellular degradation. For example, combining MAPP with labeling approaches should allow the quantification of protein degradation. In addition, one may examine how different mutations or changes in expression levels of specific proteasome subunits or regulatory components affect substrate degradation and identify changes driven by different proteasome subpopulations (e.g. constitutive versus immunoproteasome) in different biological systems and human diseases.

Further, as proteasomes are associated with many organelles throughout the cell, degradation is likely to be

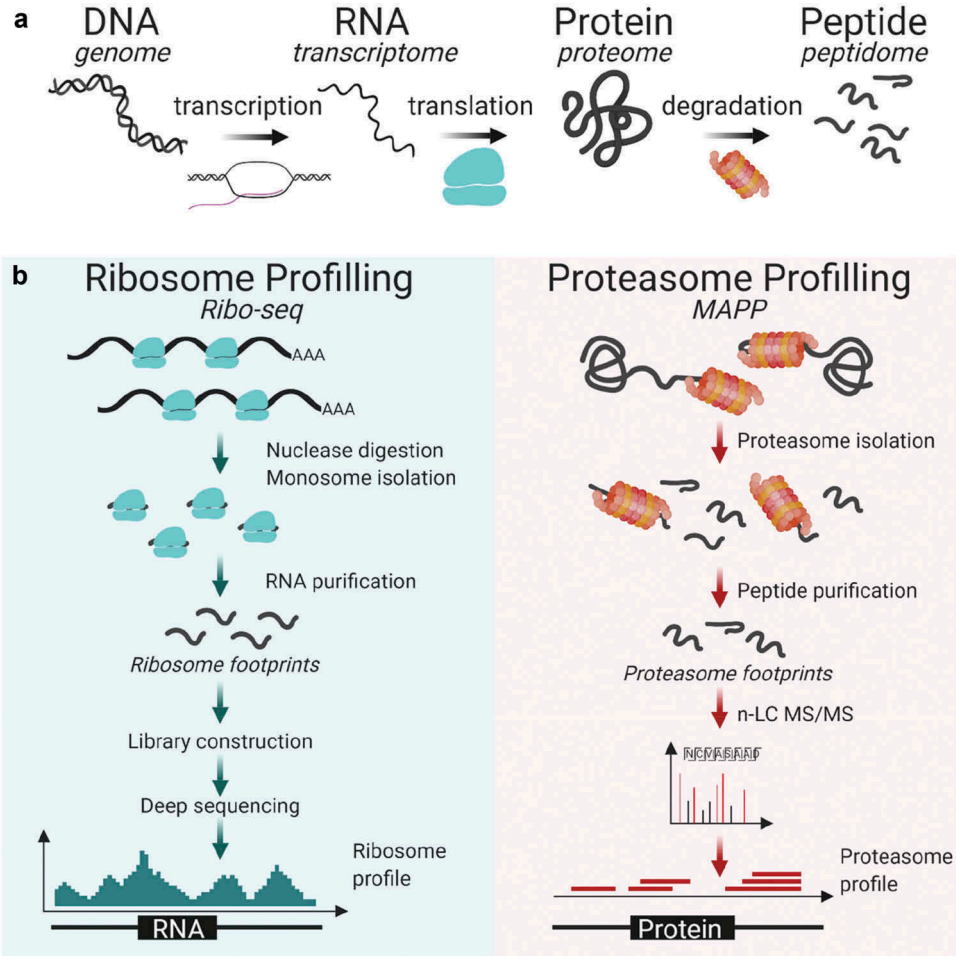

Figure 1. (a) A series of active transition steps, namely transcription, translation, and degradation regulate the balance of proteins in the cell. Currently, each of these layers of information is primarily studied through sampling and analyzing the relevant static states of the genome, transcriptome, proteome or peptidome. (b) Ribo-seq or MAPP serve to profile the active steps of translation or degradation, respectively. The two methods share conceptual underpinnings in capturing the active transitions in protein's life cycle. Both methodologies rely on the natural structure of the ribosome or proteasome to retain RNA or peptide fragments which can then be purified and sequenced. Ribo-seq captures actively translating proteins whereas MAPP identifies actively degraded proteins to reveal insight on cellular proteostasis. Figure created with BioRender.

regulated spatially and we are far from understanding regulatory principles governing these activities. Thus, Methods of analyzing substrates targeted for degradation by the proteasome will allow us to address fundamental questions regarding substrate selectivity and specificity that are key for a better understanding of the role that proteasomes play in proteostasis control.

8. Expert opinion

The complexity of the proteome emerges from the dynamic changes that proteins undergo during their life cycle across different cellular and extracellular environments. Underlying these changes are regulatory mechanisms controlling protein synthesis, protein post-translational modifications and targeted degradation, which allows fine-tuning regulation of protein function. Historically, proteomic analyses have focused on mass spectrometry-based identification of single proteins excised from gel bands or on comparative analysis of biological samples under different conditions. By the end of 2014, the first inventory list of cellular proteins across human tissues was revealed, offering for the first time a holistic view on the diversity of the human proteome [28,29]. Nevertheless, the list of proteins and their relative abundance is only a partial component in understanding proteome dynamics andthe consequences of alterations in protein function which may drive human diseases.

Recent advancements in proteomic capabilities including the sensitivity of detection, multiplexing of samples and label-free quantitation are now allowing cell biologists to start addressing questions that were not previously tractable due to limitations of mass-spectrometry instrumentation and computation. One such question is the study of protein degradation in a systematic manner. Since protein degradation provides an important protective mechanism to eliminate damaged, mutated or obsolete proteins to maintain proteostasis control, we reasoned that direct analysis of degradation landscape may reveal insight that will be different from the information provided by examining the bulk abundance of cellular proteins. Indeed, by establishing MAPP (covered herein), we highlighted degradation signatures that reflect physiological and pathological conditions, focusing on a different aspect of the life cycle of proteins. In analyzing clinical samples by MAPP we found that the set of proteins targeted for degradation were enriched for pathways that were relevant to the physiological or pathological condition. Importantly, this study provided a proof-of-concept for the ability to yield novel information from degradation profiling in clinical samples.

The recent advancements in MS technology brought about a new surge and hope in the relevance of proteomics for clinical settings due to the ability to cope with limited sample material. Numerous molecular and clinical diagnostics approaches are now being evaluated from liquid and tissue biopsies in various human pathologies ranging from neurodegeneration to cancer. However, in most of these cases, only bulk protein abundances are being measured to identify specific biomarkers indicative of disease state, as prognostic markers or as predictors for response to a specific treatment. It is therefore tantalizing to speculate whether an examination of degradation products or cleaved peptides from various types of samples (e.g. liquid biopsies and cancer tissue) would have distinct patterns, which may hold promise for biomarker discovery.

One of the biggest challenges will be to optimize the system further to allow it to provide end-point solutions for different users and make it accessible to the scientific and medical community for both basic and translational research. Heterogeneity of samples, as well as collection, processing and analysis procedures, introduces variability which may be addressed by standardized protocols and technological solutions to increase cohort size. Such efforts will undoubtedly improve the exploitation of MS-based technologies in clinical settings. This is timely and required as it is becoming clear that genomic and proteomic diversity hampers both diagnostics efforts as well as treatment efficacy of numerous therapies that are patient-specific. Inevitably, we will also gain more knowledge on the pathophysiology of human diseases from protein-based aberrations and their underlying molecular mechanisms remain to be discovered. Transforming the proteomic capabilities to generate sensitive and robust technologies should afford novel proteomics-based approaches to personalized medicine.

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Declaration of interest

No potential conflict of interest was reported by the authors.

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References

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Balch WE, Morimoto RI, Dillin A, et al. Adapting proteostasis for disease intervention. Science. 2008;319:916–919.
2. Breker M, Schuldiner M. The emergence of proteome-wide technologies: systematic analysis of proteins comes of age. Nat Rev Mol Cell Biol. 2014;15:453–464.

3. Abu-Remaileh M, Wyant GA, Kim C, et al. Lysosomal metabolomics reveals V-ATPase- and mTOR-dependent regulation of amino acid efflux from lysosomes. Science. 2017;358:807–813.
4. Kastenhuber ER, Lowe SW. Putting p53 in Context. Cell. 2017;170:1062–1078.
5. Karin M. How NF-κB is activated: the role of the IκB kinase (IKK) complex. Oncogene. 1999;18:6867–6874.
6. Bassermann F, Eichner R, Pagano M. The ubiquitin proteasome system – implications for cell cycle control and the targeted treatment of cancer. Biochim Biophys Acta. 2014;1843:150–162.
7. Dahlmann B. Role of proteasomes in disease. BMC Biochem. 2007;8:S3.
8. Rousseau A, Bertolotti A. Regulation of proteasome assembly and activity in health and disease. Nat Rev Mol Cell Biol. 2018;19:697–712.
•• **An excellent review on the crucial role which proteasomes play in governing cellular processes.**
9. Lefaki M, Papaevgeniou N, Chondrogianni N. Redox regulation of proteasome function. Redox Biol. 2017;13:452–458.
10. Wheatley DN, Giddings MR, Inglis MS. Kinetics of degradation of 'short-' and 'long-lived' proteins in cultured mammalian cells. Cell Biol Int Rep. 1980;4:1081–1090.
11. Chen X, Wei S, Ji Y, et al. Quantitative proteomics using SILAC: principles, applications, and developments. Proteomics. 2015;15:3175–3192.
12. McShane E, Sin C, Zauber H, et al. Kinetic analysis of protein stability reveals age-dependent degradation. Cell. 2016;167:803–815.e21.
• **This innovative proteomics analysis demonstrated that many proteins follow non-exponential degradation kinetics.**
13. Beaudette P, Popp O, Dittmar G. Proteomic techniques to probe the ubiquitin landscape. Proteomics. 2016;16:273–287.
14. Xu G, Paige JS, Jaffrey SR. Global analysis of lysine ubiquitination by ubiquitin remnant immunoaffinity profiling. Nat Biotechnol. 2010;28:868–873.
•• **A pioneering method by which ubiquitinated peptides are selectively purified from cellular lysates. This new approach dramatically increased the number of ubiquitnated proteins identified in a given experiment.**
15. Yau R, Rape M. The increasing complexity of the ubiquitin code. Nat Cell Biol. 2016;18:579–586.
16. Kim W, Bennett EJE, Huttlin EEL, et al. Systematic and quantitative assessment of the ubiquitin-modified proteome. Mol Cell. 2011;44:325–340.
• **Combining proteasome inhibition with diGly purification enabled the identified of proteins which are sent for degradation. This analysis identified substrates of specific ubiqutin-proteasome system enzymes.**
17. Trempe J-F, Brown NR, Lowe ED, et al. Mechanism of Lys48-linked polyubiquitin chain recognition by the Mud1 UBA domain. Embo J. 2005;24:3178–3189.
18. Collins GA, Goldberg AL. The logic of the 26S proteasome. Cell. 2017;169:792–806.
•• **A comprehensive review of the ubiquitin-proteasome system with a focus on the structure of the complex and the biochemistry of degradation.**
19. Ferrington DA, Gregerson DS. Immunoproteasomes: structure, function, and antigen presentation. Prog Mol Biol Transl Sci. 2012;109:75–112.
20. Winter MB, La Greca F, Arastu-Kapur S, et al. Immunoproteasome functions explained by divergence in cleavage specificity and regulation. Elife. 2017;6:e27364.
21. Wolf-Levy H, Javitt A, Eisenberg-Lerner A, et al. Revealing the cellular degradome by mass spectrometry analysis of proteasome-cleaved peptides. Nat Biotechnol. 2018;36:1110–1116.
•• **This new method enables the detection of peptides recently cleaved by the proteasome as well as the identity of proteins actively degraded in the cell.**
22. Larance M, Ahmad Y, Kirkwood KJ, et al. Global subcellular characterization of protein degradation using quantitative proteomics. Mol Cell Proteomics. 2012;12:638–650.
23. Dieker J, Berden JH, Bakker M, et al. Autoantibodies against modified histone peptides in sle patients are associated with disease activity and lupus nephritis. PLoS One. 2016;11:e0165373.
24. Schmidtke G, Emch S, Groettrup M, et al. Evidence for the existence of a non-catalytic modifier site of peptide hydrolysis by the 20 S proteasome. J Biol Chem. 2000;275:22056–22063.
25. Kisselev AF, Kaganovich D, Goldberg AL. Binding of hydrophobic peptides to several non-catalytic sites promotes peptide hydrolysis by all active sites of 20 S proteasomes. J Biol Chem. 2002;277:22260–22270.
26. Dallas DC, Guerrero A, Parker EA, et al. Current peptidomics: applications, purification, identification, quantification, and functional analysis. Proteomics. 2015;15:1026–1038.
27. Ingolia NT, Hussmann JA, Weissman JS. Ribosome profiling: global views of translation. Cold Spring Harb Perspect Biol. 2018;a032698.
28. Kim M-S, Pinto SM, Getnet D, et al. A draft map of the human proteome. Nature. 2014;509:575–581.
29. Wilhelm M, Schlegl J, Hahne H, et al. Mass-spectrometry-based draft of the human proteome. Nature. 2014;509:582–587.