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Decoding protein modifications using top-down mass spectrometry

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Abstract

Top-down mass spectrometry is an emerging technology which strives to preserve the post-translationally modified forms of proteins present *in vivo* by measuring them intact, rather than measuring peptides produced from them by proteolysis. The top-down technology is beginning to capture the interest of biologists and mass spectrometrists alike, with a main goal of deciphering interaction networks operative in cellular pathways. Here we outline recent approaches and applications of top-down mass spectrometry as well as an outlook for its future.

Advances in the genome sciences have generated more specific hypotheses about the dynamic nature of the protein-level language used for signaling in eukaryotic cells. This has led to an increased focus on both targeted and large-scale approaches that can precisely characterize endogenous protein forms at the molecular level. The proteomes of eukaryotic cells are highly complex owing to related genes encoding similar protein sequences and to alternative splicing and post-translational modifications (PTMs). Mass spectrometry (MS) is maturing as a powerful technology for analyzing proteomes. The emerging top-down MS approach[1-5] in particular can provide a 'bird's eye' view of the protein forms present and their relative abundances, before dissecting each for complete characterization of their primary structures. Since its early days[1,6,7], top-down mass spectrometry has continued to evolve from focused studies of single protein targets to the study of complex mixtures or even proteomes[8-10]. Here we outline the methods, applications, advantages and challenges associated with top-down mass spectrometry both for single protein targets and for entire proteomes, as well as prospects for the future.

Top-down and bottom-up MS

MS-based proteomics is typically carried out by first digesting a protein mixture into short peptides with a protease, then analyzing the peptide mixture by MS; there are many permutations on this general theme, which is conceptually known as 'bottom-up' proteomics. In contrast, the 'top-down' approach involves direct analysis of intact proteins, without previous proteolytic digestion. A new variant method, known to some as 'middle-down', analyzes larger peptide fragments (>3 kDa), thus combining some benefits of both bottom-up and top-down approaches (for example, generating peptides that contain multiple PTMs).

It is well established that protein PTMs are a key driving force behind cellular signaling. A distinct advantage of top-down over peptide-based approaches is that the abundance of the protein forms can be determined directly, as intact proteins are less susceptible to instrumental biases than are their small peptide counterparts[11]. The approach is depicted in Figure 1 for a

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hypothetical ~11 kDa protein with three forms present from three different cell states. Initially, a 'basis set' of all the forms expressed is determined for a particular cell state (Fig. 1a). Once the different forms present are detected, each form is isolated and fragmented by tandem mass spectrometry (MS/MS; Fig. 1a, inset). This process can be repeated for samples from different cell states (Fig. 1b,c, insets), which allows detection of PTM dynamics caused by genetic manipulation, drug treatment, transcript knockdown or other perturbations.

The MS/MS level of information is the top-down equivalent of the tryptic digest typically used in a bottom-up experiment. Top-down MS/MS also achieves protein identification and molecular characterization, but with a different information content. Notably, MS/MS of intact protein forms facilitates the determination of modifications as they occur in combination, as well as revealing information about PTM hierarchies (such as which PTMs occur first, second, third and so on)[11,12].

Top-down technology

The three basic pillars of MS-based proteomics are (i) the 'front end' fractionation of complex mixtures, (ii) mass spectral data acquisition and (iii) protein identification and characterization by database searching. Over the past decade, these components have improved drastically for peptides, followed by slower progress for intact proteins. Here we discuss the available approaches and technologies for top-down MS as well as the needs for specific improvements.

Front-end fractionation of intact proteins

Despite efforts toward improving 'front end' separations, no top-down platform has yet emerged as the best option. Two-dimensional gels provide a nice 'bird's eye' view of the proteome through good separation; however, large-scale analyses of gel spots by top-down MS has been difficult to achieve by either MALDI- or electrospray ionization (ESI)-based approaches. Lack of sensitivity has thwarted the MALDI approach that uses a phenomenon called 'in-source decay'[13] to achieve fragmentation and identification of intact proteins[14]. ESI is more promising for large protein MS/MS, because it generates multiply charged ions for more efficient dissociation. However, it has not yet proven possible to implement ESI in conjunction with protein electroelution from two-dimensional gels. Proteome fractionation by preparative gel electrophoresis in the first dimension using an acid-labile surfactant in place of sodium dodecylsulfate has been demonstrated in combination with ESI and Fourier-transform (FTMS)/MS[8,15].

Other common approaches involve fractionating proteins in the first dimension by anion exchange[16], capillary isoelectric focusing[17] or chromatofocusing[18]. These fractionation approaches have been implemented in work flows on diverse types of instrument, such as time-of-flight (TOF) MS[19,20] and FTMS[21]. As in bottom-up proteomics, the second dimension of separation is dominated by reversed-phase liquid chromatography (RPLC). With improved instrumentation and MS/MS methods, top-down MS should become more compatible with the timescale of chromatography used at present in bottom-up liquid chromatography-mass spectrometry (see Box 1).

MS instrumentation

Instruments become approximately fivefold more sensitive about every three years and come in basic forms such as time-of-flight (TOF), quadrupole, ion trap or FTMS, or as combinations of these. Continued refinements are needed for top-down because proteins and peptides above 5 kDa have more charge states and more isotopic peaks than the average tryptic peptide.

BOX 1

HIGH-THROUGHPUT TOP-DOWN?

Top-down MS has been quite successful for targeted studies of single, <100 kDa proteins. Extending the approach to whole proteome analysis has been challenging for several reasons. The handling of complex protein mixtures can require 1-2 orders of magnitude more material than current bottom-up analyses. For high-throughput implementation, the main limitation preventing top-down from being more competitive with bottom-up approaches is a high quality proteome fractionation that interfaces cleanly and in real time with mass spectrometry.

Only a handful of studies have reported detection or identification of as many as 100-700 proteins <70 kDa in a single study. For example, the Smith laboratory[16] reported the detection of ~700 bacterial proteins (5-40 kDa) using a one-dimensional RPLC separation with column pressures of ~8,000 p.s.i. New approaches employing the faster ion trap-FTMS hybrids with collisional dissociation are becoming available[47,48]. Recently, Chi *et al.* demonstrated MS/MS on 46 *Escherichia coli* proteins (3-20 kDa) from purified ribosomes using a new ion fragmentation method on an ion trap with a rate of spectral acquisition commensurate with that of chromatography[29]. Our laboratory has achieved high resolution MS/MS on 22 yeast proteins (11-36 kDa) from whole-cell lysates in a single liquid chromatography run using FTMS[49].

In addition, automated hardware and software dedicated to top-down approaches are currently in an underdeveloped state. As data acquisition rates increase, software tailored to interpret large top-down datasets will be needed on a high-throughput basis. Imbedding the ever-increasing number of known polymorphisms and PTMs (not just phosphorylation) into MS search engines will increase the ease with which modified proteins will be automatically and precisely characterized.

Historically, top-down MS has been most often performed on FTMS instruments, but new instruments are being developed that may facilitate tandem MS experiments for intact proteins of high mass. For example, the need for a superconducting magnet in FTMS was recently bypassed using a new approach to measuring the mass-to-charge ratio of ions[22,23]. Similar to the original type of FTMS, this new Orbitrap instrument provides high resolving power for each detected peak. This translates to low-parts-per-million mass accuracy and higher confidence in protein identification; however, both FTMS and Orbitrap instruments are slower than the lower-resolution ion trap. Even with only ~10^3 resolving power, however, the utility of the stand-alone ion traps for top-down is improving, thanks in part to new methods for fragmenting larger peptides and proteins in the gas phase. Both low and high resolution mass spectrometers should continue to drive advancements in top-down proteomics (see Box 1).

MS/MS fragmentation

The classical method for breaking ions apart involves colliding them into a neutral gas such as helium or argon, a process known as collision-induced or collisionally activated dissociation (CID or CAD). Newer methods using electrons for MS/MS are now coming into more widespread use (Fig. 2), with electron capture dissociation (ECD) first reported in 1998 (ref. 24). Recently, this concept has evolved into electron transfer dissociation (ETD) with work by the Hunt group[25,26]. Combining ETD with previous approaches for charge manipulation of ions from the McLuckey laboratory[27,28], top-down in a stand-alone ion trap is now a reality[29,30]. The electron-based methods for ion fragmentation cleave proteins and large peptides at many more backbone positions than older approaches, which translates into an improved capacity to localize modifications to specific sites. Both ECD and ETD retain any PTM that is stable during the original ionization process (Fig. 2a)[31], in contrast to collisional dissociation of tryptic peptides, which often ejects PTMs such as phosphorylation and

glycosylation (Fig. 2b). However, when applied during top-down MS/MS the classical methods of ion fragmentation frequently retain labile PTMs (Fig. 2a)[32,33]. This is likely to be due to the higher-order structure of gaseous ions larger than ~8 kDa, which drives fragmentation at amide backbone sites preferentially over PTM ejection. Another key recent advance is the ability to obtain high-quality fragmentation spectra above 200 kDa by preventing ultralarge proteins from folding up like 'spaghetti' immediately after ESI[34]. ETD and ECD will propel both top-down and middle-down strategies to achieve better protein characterization[35].

Data analysis

The specificity of database searching of intact protein MS/MS spectra is often orders of magnitude higher than that for bottom-up fragments. This translates to a greater capability to consider PTMs and coding polymorphisms (cSNPs) during a primary database search[32]. In fact, the concept of putting PTMs and cSNPs in databases[32,36] is gaining more acceptance, with an ontology for PTMs now specified by the Proteomics Standard Initiative.

The only algorithm available at present for identifying protein forms from tandem mass spectra of intact proteins is ProSight[37] (see Box 1). The standard search uses the experimental protein mass[36] to select all protein candidates that lie within a specified mass range (for example, ± 2,000 Da). ProSight then uses a Poisson model to provide statistically significant matches[32] between the fragment ion mass values in the experimental MS/MS spectrum and the theoretical masses predicted from each candidate. This type of search is error tolerant in 'Δm' mode[36], which considers the precise mass difference between the experimental protein molecular mass and the database candidate. Searching in Δm mode facilitates the detection and localization of PTMs not present in the database. ProSight uses a candidate expansion method referred to as 'shotgun annotation'[38] to consider combinations of diverse PTMs, cSNPs and alternative splicing events harbored in the database[21]. Shotgun annotation allows direct coupling of protein identification and characterization. Each year, more cSNPs and PTMs (especially phosphorylations, glycosylations and disulfide bonds) are added to public databases. The ProSight approach provides a means to access this information in new database searches.

Current applications

Chromatin biology

Histone proteins and hypotheses of a 'histone code' have attracted increasing attention over the past several years. Both top-down and bottom-up MS approaches have been very successful in generating a comprehensive map of isolated histone PTMs[11,38-43]. However, in the context of understanding the dynamic interactions and functions of these proteins, a complete view of all the PTMs present at the intact level is necessary. This kind of biological information is best obtained by performing MS/MS on intact histones or large proteolytic fragments containing many modifications[43,44]. Top-down MS reports on the combinations of methylations, phosphorylations and acetylations present on the same histone molecule *in vivo*. MS/MS fragmentation can be used to precisely identify highly similar histone variants and localize PTMs to specific sites. In addition to detecting combinations of PTMs on large fragments, top-down MS has the capability to distinguish positional isomers with the same molecular weight (such as substoichiometric acetylations on multiple lysine residues). Such 'PTM isomers' can be quantified by comparing the ratios of fragment ion abundances produced during MS/MS[11]. The overall percent occupancy of PTMs present on a particular histone can thus be estimated. Top-down MS can also precisely identify highly related histone family members—for example, the many variants of H2A[39] and H2B[40]).

Protein-level variation

In addition to PTMs, there are other sources of protein-level variation in eukaryotic cells. These include families of highly related genes encoding protein sequences with high identity (Fig. 3a, top), polymorphisms and alternative splicing (Fig. 3a, middle and right). Expression of these variations leads to a mixture of protein forms with slightly different intact masses (Fig. 3a). In addition to resolving such mixtures, top-down MS can also determine expression ratios of intact protein forms[21], unlike typical bottom-up approaches or standard RNA-level analysis. When such a mixture is analyzed by bottom-up MS, the peptides produced can either be common to all forms or isoform-specific (Fig. 3b), with the latter often difficult to detect and reassemble in the protein 'scaffold'.

Membrane proteins

Top-down MS has also made strides in the analysis of membrane proteins. In past years, substantial progress has been made in adapting this approach to integral membrane proteins (recently reviewed in ref. [45]). Fifty-eight thylakoid membrane proteins from a plant have been analyzed using these tailored chromatographic methods[46].

The future of top-down MS

Top-down mass spectrometry has made valuable contributions to our knowledge of combinations of protein PTMs. The initial contributions and measurement benefits are most clearly viewed through the lens of histone analysis in chromatin biology[44]. Continued work on histones with the top-down family of approaches will deepen our understanding of PTM dynamics in bulk chromatin and their functions in cancer and epigenetics. We anticipate that large-protein MS will continue to evolve such that sample utilization and overall speed of the measurement will approach the norms of bottom-up techniques. This will allow hundreds of top-down measurements in parallel to detect long range PTM interactions on the same protein and on separate proteins within cellular pathways.

As PTM-focused and general forms of bottom-up MS continue to mature, we project that site-specific modifications will increasingly be added to databases, streamlining the subsequent readout of complex PTM patterns and hierarchies. With improving technology, tandem mass spectrometry above 5 kDa will increasingly be applicable to cellular structures beyond chromatin, helping to advance a major goal of modern proteomics: to develop a deep sense of PTM function, how PTMs relate to each other and how they function as nodes in signaling networks.

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COMPETING INTERESTS STATEMENT

The authors declare competing financial interests: details accompany the full-text HTML version of the paper at http://www.nature.com/naturemethods/.

References

1. Kelleher NL, et al. Top down versus bottom up protein characterization by tandem high-resolution mass spectrometry. J. Am. Chem. Soc 1999;121:806–812.
2. McLafferty FW, Fridriksson EK, Horn DM, Lewis MA, Zubarev RA. Biochemistry: biomolecule mass spectrometry. Science 1999;284:1289–1290. [PubMed: 10383309]

3. Reid GE, McLuckey SA. 'Top down' protein characterization via tandem mass spectrometry. J. Mass Spectrom 2002;37:663–675. [PubMed: 12124999]
4. Kelleher NL. Top down proteomics. Anal. Chem 2004;76:197A–203A. [PubMed: 14697051]
5. Bogdanov B, Smith RD. Proteomics by FTICR mass spectrometry: top down and bottom up. Mass Spectrom. Rev 2005;24:168–200. [PubMed: 15389855]
6. McLafferty FW. High resolution tandem FT mass spectrometry above 10 kDa. Acc. Chem. Res 1994;27:379–386.
7. Kelleher NL, Costello CA, Begley TP, McLafferty FW. Thiaminase I (42 kDa) heterogeneity, sequence refinement, and active site location from high-resolution tandem mass spectrometry. J. Am. Soc. Mass Spectrom 1995;6:981–984.
8. Meng F, et al. Processing complex mixtures of intact proteins for direct analysis by mass spectrometry. Anal. Chem 2002;74:2923–2929. [PubMed: 12141648]
9. Meng F, et al. Molecular-level description of proteins from *Saccharomyces cerevisiae* using quadrupole FT hybrid mass spectrometry for top down proteomics. Anal. Chem 2004;76:2852–2858. [PubMed: 15144197]
10. Patrie SM, et al. Top down mass spectrometry of <60 kDa proteins from *Methanosarcina acetivorans* using quadrupole FTMS with automated octapole collisionally activated dissociation. Mol. Cell. Proteomics 2006;5:14–25. [PubMed: 16236702]
11. Pesavento JJ, Mizzen CA, Kelleher NL. Quantitative analysis of modified proteins and their positional isomers by tandem mass spectrometry: human histone H4. Anal. Chem 2006;78:4271–4280. [PubMed: 16808433]
12. Zabrouskov V, et al. Stepwise deamidation of ribonuclease A at five sites determined by top down mass spectrometry. Biochemistry 2006;45:987–992. [PubMed: 16411774]
13. Reiber DC, Grover TA, Brown RS. Identifying proteins using matrix-assisted laser desorption/ ionization in-source fragmentation data combined with database searching. Anal. Chem 1998;70:673–683. [PubMed: 9491752]
14. Ogorzalek Loo RR, et al. Virtual 2-D gel electrophoresis: visualization and analysis of the *E. coli* proteome by mass spectrometry. Anal. Chem 2001;73:4063–4070. [PubMed: 11569793]
15. Johnson JR, Meng F, Forbes AJ, Cargile BJ, Kelleher NL. Fourier-transform mass spectrometry for automated fragmentation and identification of 5-20 kDa proteins in mixtures. Electrophoresis 2002;23:3217–3223. [PubMed: 12298093]
16. Sharma S, et al. Proteomic profiling of intact protein using WAX-RPLC 2-D separations and FTICR mass spectrometry. J. Proteome Res 2007;6:602–610. [PubMed: 17269717]
17. Zhou F, Johnston MV. Protein profiling by capillary isoelectric focusing, reversed-phase liquid chromatography, and mass spectrometry. Electrophoresis 2005;26:1383–1388. [PubMed: 15759303]
18. Chong BE, Yan F, Lubman DM, Miller FR. Chromatofocusing nonporous reversed-phase high-performance liquid chromatography/electrospray ionization time-of-flight mass spectrometry of proteins from human breast cancer whole cell lysates: a novel two-dimensional liquid chromatography/mass spectrometry method. Rapid Commun. Mass Spectrom 2001;15:291–296. [PubMed: 11223961]
19. Zhao J, et al. Proteomic analysis of estrogen response of premalignant human breast cells using a 2-D liquid separation/mass mapping technique. Proteomics 2006;6:3847–3861. [PubMed: 16767785]
20. Nemeth-Cawley JF, Rouse JC. Identification and sequencing analysis of intact proteins via collision-induced dissociation and quadrupole time-of-flight mass spectrometry. J. Mass Spectrom 2002;37:270–282. [PubMed: 11921368]
21. Roth MJ, et al. Precise and parallel characterization of coding polymorphisms, alternative splicing and modifications in human proteins by mass spectrometry. Mol. Cell. Proteomics 2005;4:1002–1008. [PubMed: 15863400]
22. Hu Q, et al. The Orbitrap: a new mass spectrometer. J. Mass Spectrom 2005;40:430–443. [PubMed: 15838939]
23. Scigelova M, Makarov A. Orbitrap mass analyzer - overview and applications in proteomics. Proteomics 2006;6(suppl 2):16–21. [PubMed: 17031791]
24. Zubarev R, Kelleher N, McLafferty F. Electron capture dissociation of multiply charged protein cations. A nonergodic process. J. Am. Chem. Soc 1998;120:3265–3266.

25. Syka JE, Coon JJ, Schroeder MJ, Shabanowitz J, Hunt DF. Peptide and protein sequence analysis by electron transfer dissociation mass spectrometry. Proc. Natl. Acad. Sci. USA 2004;101:9528–9533. [PubMed: 15210983]
26. Coon JJ, et al. Protein identification using sequential ion/ion reaction and tandem mass spectrometry. Proc. Natl. Acad. Sci. USA 2005;102:9463–9468. [PubMed: 15983376]
27. Reid GE, Shang H, Hogan JM, Lee GU, McLuckey SA. Gas-phase concentration, purification, and identification of whole proteins from complex mixtures. J. Am. Chem. Soc 2002;124:7353–7362. [PubMed: 12071744]
28. Cargile BJ, McLuckey SA, Stephenson JL Jr. Identification of bacteriophage MS2 coat protein from *E. coli* lysates via ion trap collisional activation of intact protein ions. Anal. Chem 2001;73:1277–1285. [PubMed: 11305663]
29. Chi A, Bai DL, Geer LY, Shabanowitz J, Hunt DF. Analysis of intact proteins on a chromatographic time scale by electron transfer dissociation tandem mass spectrometry. Int. J. Mass Spectrom 2007;259:197–203. [PubMed: 17364019]
30. Amunugama R, Hogan JM, Newton KA, McLuckey SA. Whole protein dissociation in a quadrupole ion trap: identification of an a priori unknown modified protein. Anal. Chem 2004;76:720–727. [PubMed: 14750868]
31. Kelleher NL, et al. Localization of labile post-translational modifications by electron capture dissociation: the case of γ-carboxyglutamic acid. Anal. Chem 1999;71:4250–4253. [PubMed: 10517147]
32. Meng F, et al. Informatics and multiplexing of intact protein identification in bacteria and the archaea. Nat. Biotechnol 2001;19:952–957. [PubMed: 11581661]
33. Reid GE, Stephenson JL Jr. McLuckey SA. Tandem mass spectrometry of ribonuclease A and B: N-linked glycosylation site analysis of whole protein ions. Anal. Chem 2002;74:577–583. [PubMed: 11838679]
34. Han X, Jin M, Breuker K, McLafferty FW. Extending top down mass spectrometry to proteins with masses greater than 200 kilodaltons. Science 2006;314:109–112. [PubMed: 17023655]
35. Chi A, et al. Analysis of phosphorylation sites on proteins from *Saccharomyces cerevisiae* by electron transfer dissociation (ETD) mass spectrometry. Proc. Natl. Acad. Sci. USA 2007;104:2193–2198. [PubMed: 17287358]
36. Taylor GK, et al. Web and database software for identification of intact proteins using top down mass spectrometry. Anal. Chem 2003;75:4081–4086. [PubMed: 14632120]
37. LeDuc RD, et al. ProSight PTM: an integrated environment for protein identification and characterization by top-down mass spectrometry. Nucleic Acids Res 2004;32:W340–W345. [PubMed: 15215407]online
38. Pesavento JJ, Kim YB, Taylor GK, Kelleher NL. Shotgun annotation of histone modifications: a new approach for streamlined characterization of proteins by top down mass spectrometry. J. Am. Chem. Soc 2004;126:3386–3387. [PubMed: 15025441]
39. Boyne MT II, Pesavento JJ, Mizzen CA, Kelleher NL. Precise characterization of human histones in the H2A gene family by top down mass spectrometry. J. Proteome Res 2006;5:248–253. [PubMed: 16457589]
40. Siuti N, Roth MJ, Mizzen CA, Kelleher NL, Pesavento JJ. Gene-specific characterization of human histone H2B by electron capture dissociation. J. Proteome Res 2006;5:233–239. [PubMed: 16457587]
41. Medzihradszky KF, et al. Characterization of *Tetrahymena* histone H2B variants and posttranslational populations by electron capture dissociation (ECD) Fourier transform ion cyclotron mass spectrometry (FT-ICR MS). Mol. Cell. Proteomics 2004;3:872–886. [PubMed: 15199121]
42. Thomas CE, Kelleher NL, Mizzen CA. Mass spectrometric characterization of human histone H3: a bird‘s eye view. J. Proteome Res 2006;5:240–247. [PubMed: 16457588]
43. Garcia BA, Pesavento JJ, Mizzen CA, Kelleher NL. Pervasive combinatorial modification of histone H3 in human cells. Nat. Methods 2007;4:487–489. [PubMed: 17529979]
44. Taverna SD, et al. Long-distance combinatorial linkage between methylation and acetylation on histone H3 N termini. Proc. Natl. Acad. Sci. USA 2007;104:2086–2091. [PubMed: 17284592]

45. Whitelegge J, Halgand F, Souda P, Zabrouskov V. Top down mass spectrometry of integral membrane proteins. Expert Rev. Proteomics 2006;3:585–596. [PubMed: 17181473]

46. Gomez SM, et al. Transit peptide cleavage sites of integral thylakoid membrane proteins. Mol. Cell. Proteomics 2003;2:1068–1085. [PubMed: 12902551]

47. Macek B, Waanders LF, Olsen JV, Mann M. Top down protein sequencing and MS3 on a hybrid linear quadrupole ion trap-Orbitrap mass spectrometer. Mol. Cell. Proteomics 2006;5:949–958. [PubMed: 16478717]

48. Zabrouskov V, Senko MW, Du Y, LeDuc RD, Kelleher NL. New and automated MS^n approaches for top down identification of modified proteins. J. Am. Soc. Mass Spectrom 2005;16:2027–2038. [PubMed: 16253516]

49. Parks BA, et al. An online method for top down proteomics using an LTQ-FTICR. Anal. Chem. in the press

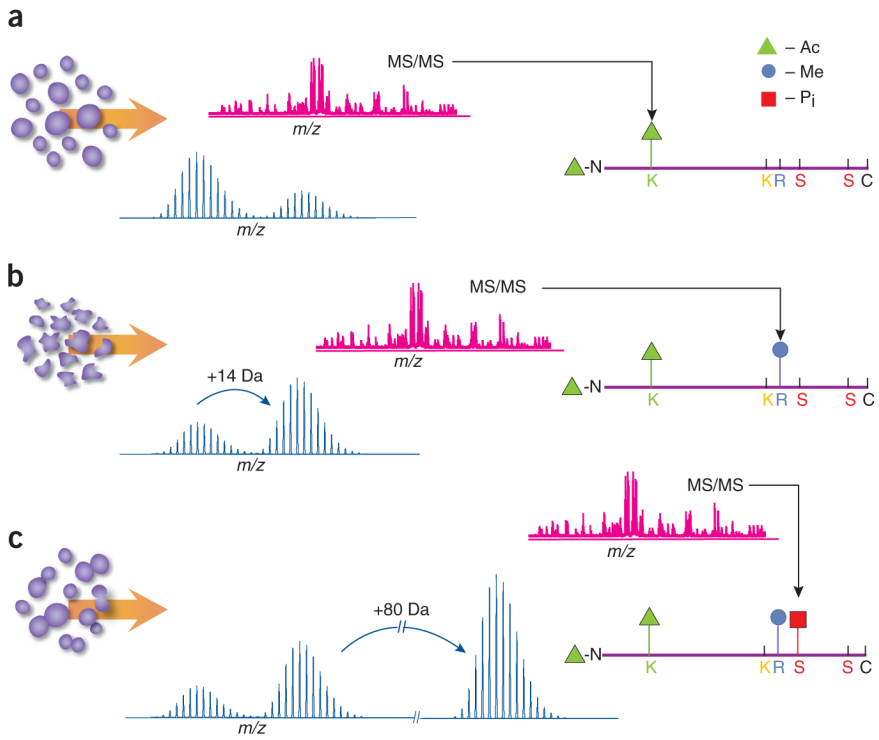

Figure 1.

The use of top-down MS for PTM detection: top-down characterization of a hypothetical protein and its PTMs from different cell states. (**a**) The protein is purified from asynchronous cells and subjected to intact MS analysis (blue trace) followed by MS/MS of individual protein forms (pink trace) to pinpoint PTM location (arrow). (**b**, **c**) The same protein target is purified from cell states of interest (for example, apoptosis (**b**) or mitosis (**c**)) and subjected to intact MS and MS/MS analysis for PTM determination. Ac, acetyl group; Me, methyl group (+14 Da); P_i, phosphate group (+80 Da).

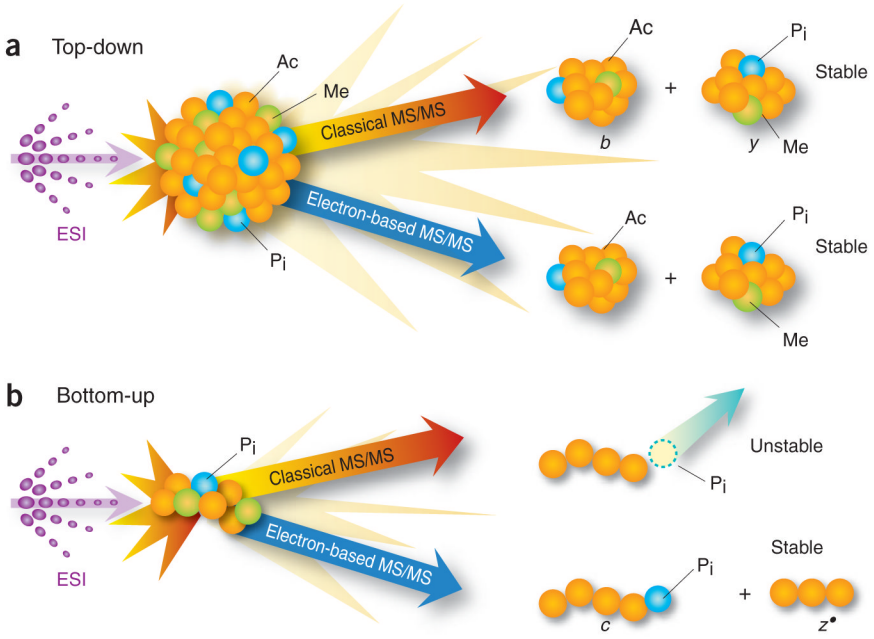

Figure 2.

Classical versus electron-based methods for fragmentation of protein and peptide ions in tandem mass spectrometry. (**a**) Most PTMs are stable during top-down fragmentation of proteins by either classical or electron-based MS/MS methods, which typically create *b*- and *y*- type or *c*- and $z^{\bullet}$-type ions, respectively. (**b**) During classical fragmentation of small peptides generated during bottom-up analysis, some PTMs such as phosphorylation are not always stable, whereas in electron-based MS/MS methods they are. Ac, acetyl group; Me, methyl group; P_i, phosphate group.

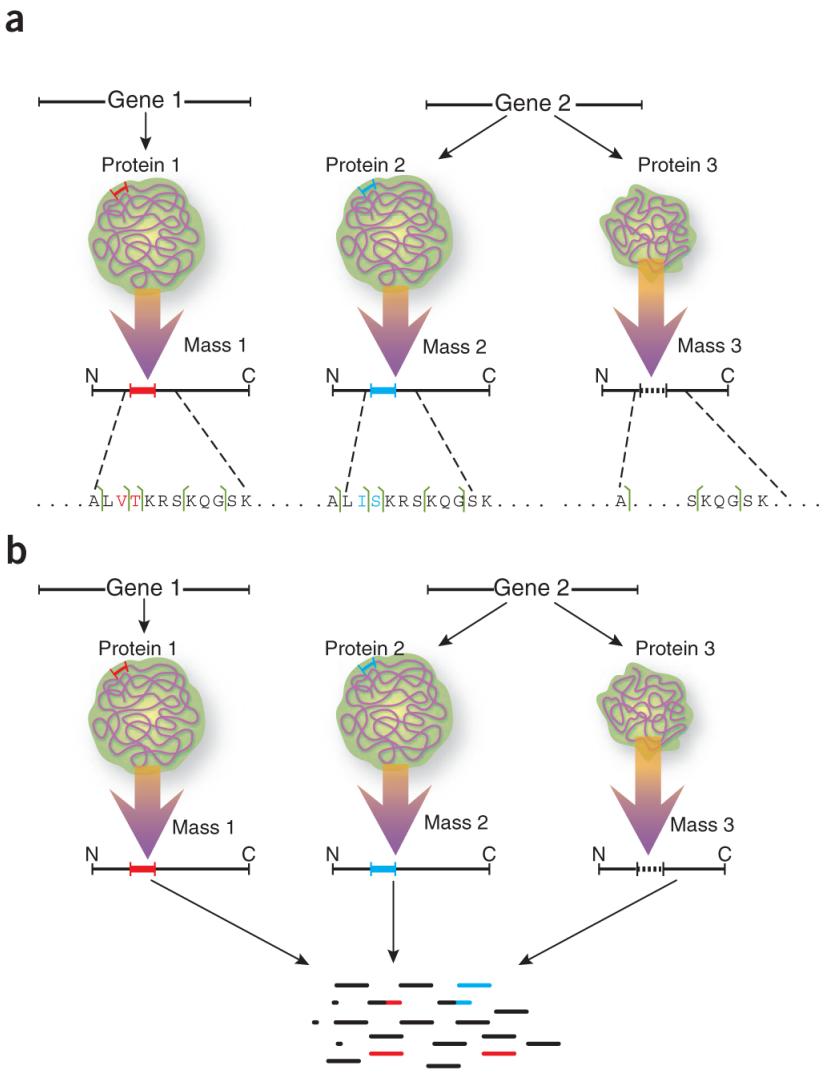

Figure 3.

The complexities of precisely characterizing eukaryotic proteins. (**a**) Top-down MS can distinguish between protein isoforms 1 and 2 (expressed from genes 1 and 2) with highly similar intact mass values based on differences in molecular mass and MS/MS fragmentation patterns. Protein forms 2 and 3 arise from alternative splicing of a single gene. The green lines indicate where MS/MS fragmentation is occurring. (**b**) Bottom-up techniques generate a mixture of peptides produced by proteolysis, making it difficult to determine to which protein each detectable peptide (red and blue) belongs.