CHEMISTRY: Mass Spectrometry: Bottom-Up or Top-Down?

Brian T. Chait, *et al.*

Science **314**, 65 (2006);
DOI: 10.1126/science.1133987

Science (print ISSN 0036-8075; online ISSN 1095-9203) is published weekly, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue NW, Washington, DC 20005.

interesting questions. How is the relative abundance of TFII-I in the cytoplasm and nucleus determined? TFII-I phosphorylation causes TFII-I translocation into the nucleus (*14*), and yet this phosphorylated form of the protein also binds to PLC-γ in the cytoplasm. Understanding precisely how these two pools of TFII-I are regulated will reveal how the two functions of the molecule are controlled. PLC-γ also plays a key role in activating many signaling enzymes, including protein kinase C, and TFII-I may regulate many of these signaling events at the plasma membrane. The existence of proteins such as TFII-I and DREAM/KChIP (*15*, *16*) that regulate both transcription and ion-channel function support an emerging paradigm whereby proteins that function both in the nucleus and in the cytoplasm of cells coordinate the overall ability of a cell to respond to membrane stimuli and to activate gene expression.

References

1. G. Caraveo, D. B. van Rossum, R. L. Patterson, S. H. Snyder, S. Desiderio, *Science* **314**, 122 (2006).
2. Y. Franke, R. J. Peoples, U. Francke, *Cytogenet. Cell Genet.* **86**, 296 (1999).
3. A. K. Ewart *et al.*, *Nat. Genet.* **5**, 11 (1993).
4. S. K. Danoff, H. E. Taylor, S. Blackshaw, S. Desiderio, *Neuroscience* **123**, 931 (2004).
5. A. L. Roy *et al.*, *EMBO J.* **16**, 7091 (1997).
6. W. Yang, S. Desiderio, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 604 (1997).
7. G. S. Bird *et al.*, *Curr. Mol. Med.* **4**, 291 (2004).
8. P. D. Wes *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 9652 (1995).
9. Y. Li *et al.*, *Nature* **434**, 894 (2005).
10. H. Nakayama, B. J. Wilkin, I. Bodi, J. D. Molkentin, *Faseb J.* **20**, 1660 (2006).
11. R. L. Patterson *et al.*, *Cell* **111,** 529 (2002).
12. D. B. Van Rossum *et al.*, *Nature* **434**, 99 (2005).
13. I. Splawski *et al.*, *Cell* **119**, 19 (2004).
14. C. D. Novina *et al.*, *Mol. Cell. Biol.* **19**, 5014 (1999).
15. W. F. An *et al.*, *Nature* **403**, 553 (2000).
16. A. M. Carrion, W. A. Link, F. Ledo, B. Mellstrom, J. R. Naranjo, *Nature* **398**, 80 (1999).

10.1126/science.1133757

CHEMISTRY

Mass Spectrometry: Bottom-Up or Top-Down?

A novel approach to mass spectrometry involving fragmentation of intact proteins in the gas phase promises to greatly improve our ability to determine protein modifications.

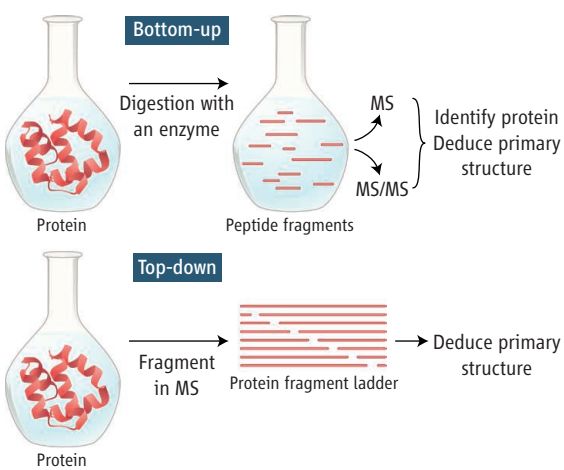
Brian T. Chait

The current revolution in proteomics and systems biology is driven by new analytical tools that are both fast and sensitive. Among these tools, mass spectrometry has become the method of choice for rapidly identifying proteins and determining details of their primary structures (*1*). Currently, there are two complementary lines of attack for the mass spectrometry analysis of proteins: the bottom-up and top-down approaches. On page 109 of this issue, Han *et al.* (*2*) extend the range of the top-down approach to proteins with molecular masses as high as 229 kD.

The bottom-up approach (see the figure, top panel) is widely used for identifying proteins and determining details of their sequence and posttranslational modifications (*1*). In this approach, proteins of interest are digested with an enzyme such as trypsin, and the resulting "tryptic peptides" are analyzed by electrospray ionization (ESI) or matrix-assisted laser desorption/ionization (MALDI). These mass spectrometry techniques allow peptide and protein molecular ions to be put into the gas phase without fragmentation (*3*). The ESI– or MALDI–mass spectrometry analyses take place in two stages. First, the masses of the intact tryptic peptides are determined; next, these peptide ions are fragmented in the gas phase to produce information on their sequence and modifications.

The bottom-up approach is especially useful for identifying proteins, because tryptic peptides are readily solubilized and separated, tasks that are considerably more difficult for the parent proteins. In addition, many tryptic peptides can be readily analyzed by mass spectrometry analysis, providing useful fragmentation ladders (*4*) that often yield sufficient information to identify the parent protein. Unfortunately, only a small fraction of the tryptic peptides are normally detected, and only a fraction of these yield useful fragmentation ladders. The bottom-up approach is therefore suboptimal for determining modifications and alternative splice variants (*5*). It is a little like having a jigsaw puzzle, where many of the pieces are missing.

But even if we had all the pieces, the picture would still be incomplete, because—to produce a sufficient number of tryptic peptide ions to allow for their detection by mass spectrometry—it is currently necessary to examine the pieces of a billion or more copies of the protein of interest. So really we have a billion jigsaw puzzles, some of which are the same, but many of which are slightly different, because they correspond to copies of the protein containing different modifications. Thus, if the pieces are relatively small (as they usually are for tryptic peptides), we will lose



Dissecting the primary structures of proteins by mass spectrometry. In the widely used bottom-up approach (**top**), proteins of interest are digested in solution with an enzyme such as trypsin, and the resulting peptides are analyzed in the gas phase by mass spectrometry in two stages. In the first (labeled "MS"), the masses of the intact tryptic peptides are determined; in the second (labeled "MS/MS"), these peptide ions are fragmented to produce information on the identity and sequence of the protein as well as its modifications. In the top-down approach (**bottom**), intact protein ions are introduced into the gas phase and are fragmented and analyzed in the mass spectrometer, yielding the molecular mass of the protein as well as protein ion fragment ladders; this information can be used to deduce the complete primary structure of the protein. Both methods make extensive use of correlations of the mass spectrometric data with protein and whole-genome sequence databases.

CREDIT: P. HUEY/*SCIENCE*

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Published by AAAS

correlations that may exist between these modifications on disparate portions of the protein. Such correlations may exist, for example, on a subpopulation of the protein that carries a phosphate moiety at two or more sites simultaneously.

In the top-down approach (see the figure, bottom panel), intact protein ions are introduced into the gas phase by ESI and are subsequently fragmented in the mass spectrometer, yielding the molecular masses of both the protein and the fragment ions. If a sufficient number of informative fragment ions are observed, this analysis can provide a complete description of the primary structure of the protein and reveal all of its modifications, as well as any correlations that exist between these modifications. Although the molecular masses of intact proteins have been successfully measured by MALDI– and ESI–mass spectrometry for some time (*3*), it has proved difficult to produce extensive gas-phase fragmentation of intact protein ions, especially from large proteins.

Han *et al.* now demonstrate that they can obtain highly informative fragmentation for proteins with molecular masses extending to more than 200 kD. The authors achieve this remarkable feat by pumping relatively large amounts of energy into the ionized protein throughout the ion injection and collisional dissociation steps, apparently maintaining the protein in an unfolded and conformationally uncollapsed state. In so doing, they considerably improve the prospects for the top-down approach.

Together with the recent introduction of two other highly effective methods for fragmenting large peptides and proteins—electron capture dissociation (*6*) and electron transfer dissociation (*7*)—this critical fragmentation component of the top-down approach now appears within reach. However, other formidable challenges remain to be overcome before the top-down approach can be considered truly robust for proteomics studies, rather than a technique for studying single purified proteins.

One major challenge is the need to separate small quantities of complex mixtures of proteins prior to mass spectrometric fragmentation. The distinctly different physico-chemical properties of different proteins make them difficult to handle as mixtures without incurring overwhelming losses of certain components or rendering the proteins incompatible with ESI-mass spectrometry. This problem has been successfully addressed with sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), because the association of the detergent SDS with proteins tends to nullify their individual properties. Unfortunately, the presence of ionic detergents, such as SDS, is not compatible with ESI, and this option is therefore not open to top-down proteomic studies. Other possibilities that are compatible with ESI–mass spectrometry include chromatography in agents that keep a wide range of proteins in solution (*8*), separations within the mass spectrometer based on mass (*9*) or ion mobility (*10*), or combinations of these methods.

Equally challenging is the need to separate slightly different forms of the same protein that differ as a result of modifications and in vivo proteolytic processing. Sensitivity is also a major challenge, because effective fragmentation of a high-molecular-mass protein implies that the protein will break up in a very large number of different ways. Thus, the intensity of any given fragment will be weak compared to that from small low-molecular-mass peptides.

Despite these challenges, it seems likely that the bottom-up and top-down approaches will continue to coevolve. Perhaps they will initially meet halfway as a hybrid approach, in which large fragments or whole domains of proteins are analyzed intact. Ultimately, developments such as those described by Han *et al.* should allow us to analyze and describe in detail the complete primary structures of proteins on a proteomic scale.

References and Notes

1. R. Aebersold, M. Mann, *Nature* **422**, 198 (2003).
2. X. Han, M. Jin, K. Breuker, F. W. McLafferty, *Science* **314**, 109 (2006).
3. B. T. Chait, S. B. H. Kent, *Science* **257**, 1885 (1992).
4. A fragmentation ladder is a series of peptide fragments with a common terminus, but differing in length on the opposite side of the peptide.
5. Alternative splicing of a pre-mRNA that is transcribed from one gene can result in the production of different mature mRNA molecules and ultimately in the translation of related but different proteins, i.e., alternative splice variants.
6. R. A. Zubarev *et al.*, *Anal. Chem.* **72**, 563 (2000).
7. J. J. Coon *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 9463 (2005).
8. R. W. Wozniak, G. Blobel, M. P. Rout, *J. Cell Biol.* **125**, 31 (1994).
9. H. Hernandez *et al.*, *EMBO Rep.* **7**, 605 (2006).
10. R. A. Sowell *et al.*, *J. Am. Soc. Mass Spectrom.* **15**, 1341 (2004).

10.1126/science.1133987

EVOLUTION

Fossil Record Reveals Tropics as Cradle and Museum

Charles R. Marshall

Over the past 11 million years, most bivalves that originated in the tropics expanded their ranges out of the tropics, where they now dominate the living extratropical fauna.

Most groups of organisms show a pronounced decrease in biodiversity from the tropics to the poles. Understanding this long-recognized latitudinal biodiversity gradient requires unraveling the evolutionary dynamics behind it. Typically, work has centered on whether the tropics have unusually high origination rates (in which case they are a cradle of biodiversity), or unusually low extinction rates (in which case they represent a museum of biodiversity). On page 102 of this issue, Jablonski *et al.* (*1*) add a new wrinkle to understanding the evolutionary dynamics of latitudinal diversity gradients by showing that much of the diversity of bivalves outside of the tropics is driven by the expansion of the geographic ranges of species that originated in the tropics. Thus, they argue that the tropics are both a cradle of biodiversity and a museum.

Most studies ignore the possible role of migration in latitudinal diversity gradients. Jablonski *et al.* report the first comprehensive analysis of the fossil record to document the patterns of origination, extinction, and migration. With a meticulously standardized taxonomy, they analyzed the fossil record of 163 genera and subgenera of bivalve mollusks that originated since the beginning of the late Miocene, 11 million years ago.

However, using the fossil record is not straightforward. Jablonski *et al.* had to overcome the relatively poor fossil record of the tropics. The lack of outcrop, the deep weathering of tropical rocks, and the dearth of research effort in the tropics have led to the recovery of, at the very least, 25 times as many bivalve fossils from the extratropics as from the tropics [see note 43 in (*1*)]. Thus, even if a

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