Top-down mass spectrometry

Top-down mass spectrometry offers the ability to sequence intact proteins—post-translational modifications and all—but is not yet a high-throughput method.

As the well-established 'bottom-up' mass spectrometry–based approach continues its success in high-throughput proteomics, an emerging approach known as 'top-down' is beginning to make headlines, especially for the analysis of post-translational modifications (PTMs).

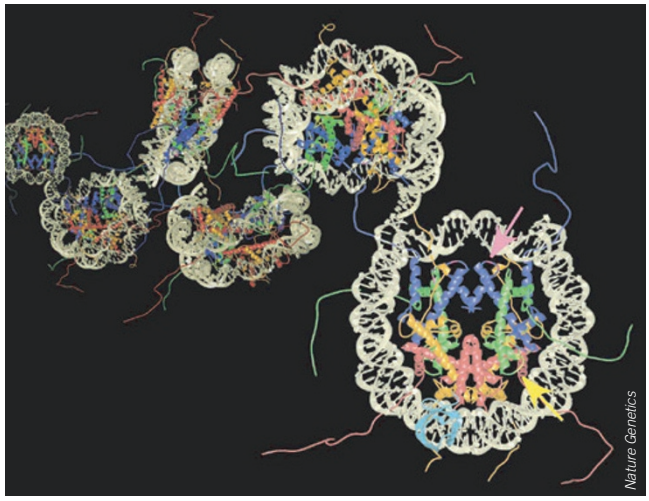
PTMs often occur in different combinations on individual proteins, and understanding these combinations is crucial for understanding biological regulation, such as for 'cracking' the histone code. In a bottom-up experiment, the proteomic mixture is digested into short peptides before analysis, so information about the correlated relationships of different PTMs is lost. Compounding the problem is the fact that many PTMs are unstable under typical mass spectrometry conditions, and that the mass spectrometer does not detect every last peptide, so minor PTMs occurring on a small percentage of proteins are often not observed.

In the top-down approach, intact proteins are introduced into the mass spectrometer, so important information about combinatorial PTMs is retained. In recent years, highly efficient fragmentation methods have been developed (such as electron capture dissociation and electron transfer dissociation) that are particularly good at preserving labile PTMs. The mass range of top-down has been extended to proteins as large as 229 kDa (*Science* **314**, 109–112; 2006), and increasingly larger numbers of intact proteins can be detected in a single analysis. Yet top-down is still mainly a technique for analyzing single purified proteins.

Currently, larger sample quantities are required and the analysis time is longer than for a bottom-up experiment, precluding high-throughput analyses. New methods are needed for efficient protein separation, and robust computational tools for assigning protein identities and PTMs from top-down data are also lacking.

So stay on the lookout for new methods driving the limits of top-down mass spectrometry. Perhaps one day this approach will be the method of choice for investigating the biological importance of combinatorial PTMs.

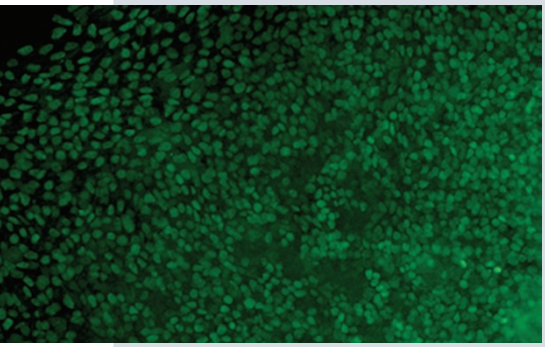
Allison Doerr



Deciphering the histone code could be aided tremendously by high-throughput top-down mass spectrometry.

Induced pluripotency in human cells

Differentiated cells can be reverted to an 'embryonic' state. But how embryonic are they really?



Human embryonic stem cells expressing a pluripotency marker.

The process of development—in which a single-celled zygote changes into a multicellular organism—should be, at least in principle, reversible. In the most recent practical demonstration of this prediction, several research groups have shown that small sets of genes, when introduced into somatic cells, can render these cells pluripotent, or able to generate all cell types—in a manner of speaking, make them young again.

The stage was set in 2006 and early 2007 with work in the mouse. Most recently, the groups of Shinya Yamanaka at Kyoto University and James Thomson at the University of Wisconsin–Madison have induced pluripotency in human skin cells by expressing a combination of just four genes.

These induced pluripotent stem (iPS) cells share several important features with human embryonic stem (hES) cells, the now classic example of a pluripotent cell type. iPS cells have similar patterns of gene expression to embryonic stem cells, they display key morphological and genetic markers, and most importantly, like stem cells, they can give rise to all three major tissue lineages *in vitro* and, in grafts into mouse, *in vivo*. So iPS cells may in the future serve as disease- or person-specific research models but, and in this respect they are distinct from stem cells, without the need for reprogramming by nuclear transfer into human oocytes.

But how similar are iPS cells to hES cells? Are the two reported sets of genes the only ones that can induce pluripotency? Can these cells be created without the use of oncogenes and retroviruses, making future therapeutic use more likely? Can the efficiency of the induction process, extremely low at present, be improved? What will this tell us about the still-mysterious nature of reprogramming and of animal development? Watch for progress in this area in the future.

Natalie de Souza

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