The era of 'omics unlimited

Raj P. Kandpal, Beatrice Saviola, and Jeffrey Felton
Department of Basic Medical Sciences, College of Osteopathic Medicine of the Pacific, Western University of Health Sciences, Pomona, CA, USA

BioTechniques 46:351-355 (April 2009 Special Issue) doi 10.2144/000113137
Keywords: human genome; post-genomic era; genomics; transcriptomics; proteomics; interactomics; molecular imaging

Determining the primary sequences of informational macromolecules is no longer a limiting factor for our ability to completely understand the biological functioning of cells and organisms. Similarly, our understanding of transcriptional regulation (transcriptomics) has been greatly enhanced by the availability of microarrays. Our next hurdle is to learn the biochemical functions of all the gene products (proteomics) and the totality of all the interactions among them (interactomics). Using traditional biochemical methods, this will take a very long time. More efficient methods are needed to address these questions, or at least to suggest possible candidates for further testing. High-resolution imaging using molecule-specific tags will reveal details of cellular architecture that are expected to provide additional insights and clues about the interactions and functions of many gene products. Computer modeling of macromolecular structures and functional systems will be of key importance. We present here a brief historical and futuristic perspective of genomics and some of its other 'omics offshoots in the post-genomic era.

Genomics revolution and post-genomic era

The importance of determining the entire genome sequence of humans was recognized more than two decades ago and was an important first step in ushering the field of genomics. It was also apparent at that time that the goal of deciphering the complete human genome was achievable with existing sequencing techniques (1–3) and by developing large-scale cloning and mapping strategies. Therefore, the human genome sequencing enterprise began by first characterizing markers to assemble a linkage map and developing a physical map of the genome. The assembly of a genetic map was greatly expedited by the availability of well-characterized markers uniformly distributed along the entire genome. The use of rare-cutting restriction enzymes and the analysis of large DNA fragments by pulsed-field gel electrophoresis facilitated the construction of a physical map. The development of vectors capable of accommodating large genomic fragments allowed the assembly of the human genome as a tiling array of large-insert clones (4). These larger insert clones were further supplemented with cosmid and phage lambda clones of genomic DNA. Using the Sanger technique of dideoxy chain terminators that were fluorescently tagged, the sequencing enterprise began with an international consortium funded by the Department of Energy, and was continued by the Human Genome Project at the National Institutes of Health (NIH) in the United States. Simultaneously, a competing effort was led by J. Craig Venter of The Institute for Genomics Research (TIGR) and Celera Genomics. While the NIH-led consortium took advantage of the tiling array of ordered clones to sequence the genome, the Venter group employed a random shotgun sequencing method which used computer analysis to assemble the overlapping sequences. With both groups adopting different approaches, the draft human genome sequence was completed ahead of schedule in 2001 (5,6).

The next step in this direction has been to study human genetic diversity by identifying different haplotypes present in the population by sequencing individual representative genomes (7). In the course of this overall undertaking, new DNA sequencing technologies have repeatedly been developed, such as pyrosequencing and single-molecule sequence-by-synthesis. These methods have made sequence analysis faster and cheaper, so that the goal of sequencing an entire human genome for $1000 will be achieved in the near future, and a single prokaryotic genome sequence will cost about $1. In the post-genomic era, the advent of methods for simultaneous evaluation of a large number of transcripts (transcriptomics), RNAi/miRNAs (interferomics/microRNomics), proteins (proteomics), interacting proteins (interactomics), DNA and chromatin modifications (epigenomics), and metabolites (metabolomics) have made the description of a comprehensive model of life achievable.

Transcriptomics

Following the sequencing of the human genome, the task at hand was to determine the genes that the sequence represents. The earliest estimates were that the human genome codes for over 100,000 transcripts, collectively known as the transcriptome. However, these estimates underwent considerable revisions until bioinformatic approaches predicted the number of transcripts encoded by the genome to be 20,000–25,000 (8), which is only about four times that for the bacterium Pseudomonas (9,10). It warrants mention that all of these genes are not expressed in every cell. The next wave of investigations then focused on which genes are expressed in a particular cell/tissue type or on a comparative analysis of transcripts between two conditions or cell/tissue types. These analyses took advantage of the human genome sequence and the knowledge of expressed sequences either from open reading frame (ORF) and intron/exon analysis, or from sequencing cDNAs cloned from various tissues. Oligonucleotides corresponding to every ORF were arrayed onto a high-density slide or chip, and these microarrays were then hybridized with target cDNA (or cRNA) generated from RNA isolated from a cell-

or tissue-type (11,12). These studies have given deeper insights into changes in gene expression as a function of a disease and/or developmental stage.

MicroRNomics

It appears that approximately one-third of the transcripts in an animal cell may be controlled by microRNAs (miRNAs). The profiling of miRNAs (13) has helped explain the significance of animal cell transcriptomes. The new paradigm of RNA interference (RNAi) proposed in 1998 added an additional layer to the regulation of gene expression in an animal cell (14). The RNAi phenomenon clarified the regulatory role of non-coding RNAs or miRNAs, and these small RNAs have come to occupy an important place in the hierarchy of gene regulation (15,16). Although array-based platforms exist for global profiling of miRNAs (microRNomics) in a cell or tissue, the significance of a specific profile is difficult to explain, because there are hundreds of target sequences in the human genome that may be potentially regulated by a specific miRNA. Newer experimental or bioinformatic methodologies will evolve in the near future to allow high-throughput identification of the most likely target of a specific miRNA in a particular cell or tissue under a given developmental stage, nutrition, environmental, or disease status.

A huge challenge for cell/molecular biologists is to determine the structure and function of the translated products of all the genes encoded in the human genome. Although there are 20,000–25,000 genes in the genome, alternative splicing of these genes leads to multiple transcripts for a given gene, and these alternatively spliced transcripts then lead to protein products which may have a related, opposite, or an entirely different function. The initial studies on proteomics started by characterizing simpler organisms such as a prokaryotic cell (*Escherichia coli*) or a unicellular eukaryotic cell (yeast) and analyzing the proteome using two-dimensional (2-D) gel electrophoresis. Specific spots were identified by using protein fragmentation, followed by separative techniques in tandem with mass spectrometry. The comparisons between two cell types or two conditions necessitated separation of proteins in parallel on two gels. However, newer techniques such as difference gel electrophoresis (DIGE) have made it possible to use a single gel to perform electrophoretic separation of a mixture of several protein samples that each have different fluorescent labels incorporated in their proteins (17). Imaging of the gel for the specific fluorescent tags and merging of images can allow assessment of relative abundances of individual proteins in different treatments, disease states, and developmental stages of cell/tissue types. The underlying 2-D electrophoresis, although technically simple, adds a time-consuming step to this process. This requirement has been eliminated by using the iTRAQ (isobaric tag for relative and absolute quantitation) procedure, which incorporates an isobaric tag with different molecular weight reporters into proteins, followed by capillary electrophoresis in tandem with mass spectrometry (18). This methodology has been used to determine the composition of proteins in a multimeric complex; specifically, the interacting proteins or complexes of a signaling network. Although informative, these basic tools by themselves are not sufficient to answer a variety of cell biology questions. It would be valuable to generate a high-density microarray containing all the proteins encoded by every individual ORF in the genome, and also another microarray containing one or more antibodies, Fab fragments, or single-chain variable fragments (scFv) against every one of these proteins. These arrays could be used for rapid profiling of proteins and interacting sets of proteins.

The above analyses do not address the N-terminal processing, phosphorylation, methylation, acetylation, glycosylation, and ubiquitination status of individual proteins. Therefore, methods need to be expanded to investigate protein modifications. The difficulty of characterizing modifications to protein can be potentially overcome by developing methods for retaining modified proteins or protein complexes onto antibody beads that are amenable to high-throughput sequencing by mass spectrometry. The antibodies can be potentially made against all modified residues in the context of recognition motifs found in the protein primary sequence.

We intuitively feel that the 20,000–25,000 genes of our genome are not enough to account for the complexity of the human organism. Even if we count all the protein domains and the products of alternate splicing, it still appears insufficient. To achieve the observed complexity from our very limited genome, an organism would have to utilize a molecular trick, and that trick would probably be the same one employed by the adaptive immune system, namely combinatorial complexity (in this case involving only protein association). Therefore, to elucidate the complete functioning of an organism, we not only need to learn the biochemical function(s) of every protein and every domain, we also need to discover all the protein-protein interactions. We need to map out what has been called the interactome.

Interactomics and relevant strategies

Such interactions have been investigated using the yeast two-hybrid assay (19) and have been compared between humans and other species (20). In another method, protein complexes involved in large signaling networks have been isolated by co-immunoprecipitation, followed by identification of the proteins comprising the network (21). These studies have allowed placement of proteins in a functional network. Other methods applicable to questions of protein-protein interaction are chemical cross-linking, affinity chromatography, and other binding studies. High-throughput versions of some of these methods have already been developed (22,23), although room for further improvement remains. Perhaps some of these methods can be combined with other techniques to create a more powerful version of the method. Microarrays and mass spectrometry come to mind as possible examples of such power-enhancing modifications. Groups of proteins collected by their interaction with one index protein could be identified by incubating them with a whole-proteome antibody microarray to see what else lights up. A useful strategy that might be exploited is to obtain protein lysates from cells that have been treated with reversible cross-linking agents, fractionate the mixture by molecular weight on gel filtration columns, unlink the cross-linked proteins, and finally, identify the individual proteins by mass spectrometry. It would be beneficial if this protocol could be automated. Another strategy that appears promising is the use of fluorescence resonance energy transfer (FRET) approaches that would allow sequential interaction among a suspected set of proteins (24). In this method, individual proteins would be tagged with either a donor or an acceptor fluorophore that have compatible emission and excitation spectra so that energy emitted from the donor fluorophore tag on one protein can excite the acceptor fluorophore on its interacting partner. We envision that the availability of an expanded set of energy-appropriate tag molecules may enhance the power and utility of FRET for the study of the interactome. In principle, such strategies may pave the way for us to understand the protein network in the structural context. These strategies can also enable us to analyze networks containing

large numbers of proteins (>180 proteins in the NMDA receptor complex) or even much larger numbers in the post-synaptic density of neurons (25,26). We also have the use of suppressor mutations (not tRNA suppressors) to identify functionally related genes, but this approach is experimentally laborious.

The analysis of interacting protein complexes composed of membrane proteins poses a special problem. The membranes must be disrupted in order to release the proteins so that their continued interaction can be revealed, but it is likely that the protein complexes themselves might also be disrupted if the methods used are too harsh. A potential remedy to this problem is to stabilize the membrane protein complexes by chemical cross-linking prior to disrupting the membranes.

Methods for detecting protein-protein interaction would have to be optimized to minimize the background, and even then non-specific binding might be a problem. But if different methods all pointed to the same interactions, that would lend credence to the results. The high-throughput methods could point the way toward candidate interactions that could be further tested using better methodology.

Bioinformatic approaches exist to determine putative structural interactions between proteins, based on the structural motifs and domains/folds in proteins (27–29). These in silico interactions can be confirmed by co-immunoprecipitating protein complexes and by the knowledge of their placement in pathways. There has been considerable progress in predicting the 3-D structures of proteins based on the primary sequence (27,30). The comparison of in silico 3-D structures among themselves or with experimentally determined structures may suggest assignment of these proteins to known networks. The final evidence for these interactions could be validated by performing in vivo interactions of molecularly cloned and expressed proteins in bacterial or mammalian cells. A strategy that will be helpful for experimental verification of these interactions is to transfect host cells with pairs of individually tagged expression constructs and to perform molecular imaging of these cells to determine the nearest neighbors of a protein. If such complexes could be identified, then one could potentially describe a set of nodes in the cellular architecture that are interlinked.

Experimental advances

Methods need to be refined for investigating the biological relevance of a portion of the interactome and its significance. The existing methods of tissue-specific knockout using the cre-lox strategy can potentially be expanded in a high-throughput manner, so that the relevance of specific nodes of the interactome can be linked to biological phenotypes. We believe the potential of proteome and interactome studies can be better harnessed by prioritizing them on the basis of biological complexity and in the context of human health and disease. These strategies would also allow us to recognize a topographical arrangement of proteins in the cellular milieu by using specific molecular tags. Some of these tags would be covalently linked and other tags would have been developed on the basis of their ability to bind to a specific protein by structural affinity; potentially such molecules would be small molecular weight peptides with heavy metal or fluorescent tags. Such individual protein-specific stains might be rationally designed by computer and produced by automated synthesis. This in turn would require perfected algorithms for prediction of secondary and tertiary structure of proteins. It may not be too long before imaging techniques mature to allow a glimpse of the molecular architecture of proteins inside the cell that would include the localization of individual proteins in the cytosol or organelles, their position in membranes, and their proximities to other proteins.

Visualizing single molecules within cells is now possible. Electron microscopy has been important in the past to image individual proteins, although it relies on fixation of tissue or cells. New advances in electron cryotomography can image cells in 3-D in a nearly native state (31,32). This method is useful to peer into a cell, provided the cross-section is not too thick. This method is being used to investigate the molecular structure of cells and to elucidate the architecture of large protein assemblies such as protein motors and the cytoskeleton. Techniques such as near-field scanning optical microscopy (NSOM), which employs a high-resolution ultra-fine scanning probe combined with fluorescence detection, permit improved image resolution on unfixed tissue specimens (33,34). This technique has been used for detecting individual proteins and their conformations, specifically on the accessible cytoplasmic membrane (35,36). In the past, light microscopes have been limited to visualizing molecules that are approximately one-half the wavelength of light used to detect them, which constitutes the diffraction limit. Advances in light microscopy have challenged that paradigm. Far-field optical super lenses and photoswitchable proteins can decrease the diffraction limit (37–39). In addition, it is now possible to produce detailed 3-D images by computationally improving the limit of resolution (40). Increased advances in this area and increased ability to visualize molecules within the cell and on the cell surface should facilitate our ability to determine which proteins are interacting within the cell and where those interactions are occurring.

Molecular imaging

Such techniques to improve the resolution of microscopy would be a step toward a complete description of the 3-D architecture of the cell at the 10-nm level. However, the really valuable information would be to put this structure in the context of the interactome. It is not enough to be able to image structures if we do not know the molecular identities of the structures we are seeing. We need to know what we are seeing and what is next to what. For things like microtubules and nanomachines, the identities may be obvious, but for average-sized globular proteins, one looks much like another.

Knowing the structure and function of networks at this level would be likely to provide clues that would feed back and help us figure out the biochemical functions of the unidentified components. Annotation has severe limits. Just because bioinformatics tells us that a protein is probably an esterase, a serine protease, or a protein kinase does not mean we know what the protein is really doing in the cell. Knowing the interactions and architecture will help us gain that understanding and will suggest further experiments.

We now have knowledge of the genetic and transcriptional information of many organisms and some knowledge of protein-protein interactions under various conditions. We are at the point of applying that knowledge in a more fundamental manner. It is essential to understand not only what proteins are produced, but also what their functions are and where they are ultimately located within the cell. Analyzing and cataloging cellular locations of proteins under various environmental or developmental conditions will be of great importance. Software could be developed that would incorporate this information and model the functioning of cells. Such modeling would take into account which proteins are produced in each particular environmental condition or developmental stage as well as protein-protein interaction data. Such a model would also utilize direct experimental data of the location of proteins within cells. Thus, a global model could be built to simulate the workings of the cell. Inputting a perturbation of the system (for example, decreasing the level of one protein) would result in a modeled outcome that could be tested experimentally. This type of predictive system would aid in the basic understanding of cellular processes and in the development of chemotherapeutics to ameliorate disease processes. Predictive modeling would also improve

decision-making processes. With so many potential cellular pathways to investigate, narrowing the choices to those that would likely bear the most scientific fruit would thus enable investigators to focus resources on lines of experimentation with the highest likelihood of success.

Future outlook

In summary, it is anticipated that when the science of proteomics merges with cell biology, we will a have better understanding of how different cellular processes are regulated. We predict that the proteomic field will move rapidly in the next decade to develop newer methods for describing abundance, localization, modification, and changes in the topographical arrangement of proteins in cells. The methodology will allow comprehensive analysis and comparison of the proteome/interactome of different cell types/tissues. Strategies will emerge to visualize real-time changes in the proteome of a single cell by using a variety of tag molecules. By the time *BioTechniques* is ready to again address the future of methods in molecular biology, it is hoped that many of the grandiose plans proposed herein will have been accomplished.

Acknowledgments

The authors declare no competing interests.

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Received 10 February 2009; accepted 26 February 2009.

Address correspondence to Jeffrey Felton, Professor of Microbiology, Department of Basic Medical Sciences, College of Osteopathic Medicine of the Pacific, Western University of Health Sciences, 309 E. Second Street, Pomona, CA, 91766. email: jfelton@westernu.edu