# Proteomics: Applications to the Study of Rheumatoid Arthritis and Osteoarthritis

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## Abstract

The study of both DNA and protein technologies has been marked by unprecedented achievement over the last decade. The completion of the Human Genome Project in 2001 is representative of a new era in genomics; likewise, proteomics research, which has revolutionized the way we study disease, offers the potential to unlock many of the pathophysiologic mechanisms underlying the clinical problems encountered by orthopaedic surgeons. These new fields are extending our approach to and investigation of the etiology and progression of musculoskeletal disorders, notably rheumatoid arthritis and osteoarthritis. Advances in proteomics technology may lead to the development of biomarkers for both rheumatoid arthritis and osteoarthritis. Such biomarkers would improve early detection of these diseases, measure response to treatment, and expand knowledge of disease pathogenesis.

Rheumatoid arthritis (RA) and osteoarthritis (OA) are two of the most common chronic musculoskeletal disorders worldwide.[1] A survey conducted by the American Academy of Orthopaedic Surgeons reported that 7.3 million orthopaedic procedures were performed in US hospitals in 1995. Of these, OA and back pain were the most commonly treated problems. Musculoskeletal disorders as a whole account for $215 billion each year in health care costs and loss of economic productivity.[2]

Less common than OA, RA affects 1% of the population worldwide.[3,4] Although the long-term prognosis for RA likely will improve with new pharmacologic therapies, the disease remains a difficult problem. Average life expectancy of affected patients is reduced by 3 to 18 years, and 80% of patients are disabled after 20 years.[5,6] On average, the annual cost of each case of RA in the United States is approximately $6,000.[6] Although contemporary drugs are effective, our ability to diagnose RA with a high degree of sensitivity and specificity remains limited. The development of a diagnostic assay—the identification of a biomarker for RA—would enable the delivery of new effective therapies earlier in the disease stage, possibly before signs of joint destruction manifest. Despite the many advances in our understanding of the pathophysiology of both RA and OA, identifying the etiology of these disorders continues to be elusive.

We are, however, in the midst of a revolution in research design, tech-

**Table 1**

**Glossary of Terms**

| Term | Definition |
|---|---|
| Proteome | The profile of all proteins expressed in the extracellular and/or intracellular environment. |
| Proteomics | The identification, characterization, and quantification of all proteins involved in a particular pathway, organelle, cell, tissue, organ, or organism that can be studied to provide accurate and comprehensive data about that system. |
| Yeast two-hybrid assay | An experiment that studies protein-protein interactions in a semi–in vivo system. It involves the subcloning of the genes of the proteins in question into vectors with a portion of a transcriptional activator of a reporter gene. |
| Mass spectrometry | A technique that produces and measures, usually by electrical means, a mass spectrum. It separates ions according to the ratio of their mass to charge, allowing the abundances of each isotope to be determined. |
| Mass spectrometry–based proteomics | A technique currently dominated by the analysis of peptides originating either from digestion of proteins separated by two-dimensional gel electrophoresis or from global digestion. The simple peptide mixtures obtained from digestion of gel-separated proteins do not usually require further separation, whereas the complex peptide mixtures obtained by global digestion are most frequently separated by chromatic technique. |
| Edman degradation | Cyclic degradation of peptides based on the reaction of phenylisothiocyanate with the free amino group of the *N*-terminal residue, such that amino acids are removed one at a time and identified as their phenylthiohydantoid derivatives. |
| Epitope | A unique molecular shape or sequence carried on a microorganism that triggers a specific antibody or cellular immune response. |

niques, and capabilities. Proteomics, the large-scale analysis of proteins, is emerging as a field that holds great promise for unlocking many of the pathophysiologic mechanisms of disease (Table 1).

## Development of Proteomics

Over the past 25 years, high-throughput sequencing of DNA has revolutionized the way we view disease and conduct biomedical research. With the development of the polymerase chain reaction and the automated DNA sequencer, as well as with the completion of the Human Genome Project, the high-throughput, large-scale approach has become a clear requisite to understanding the complex pathophysiologic mechanisms underlying human diseases. High-throughput analysis of DNA using sequencing techniques, DNA microarrays, and cellular and molecular biology has formed the foundation of genomics.

However, the accumulation of enormous amounts of DNA sequence data does not necessarily translate into an understanding of biologic function. In fact, there is no absolute correlation between gene expression via messenger RNA and protein end products.[7] Proteomics thus is complementary to genomics because of its focus on the identification and characterization of gene products (ie, proteins). Proteomics is the necessary next step for biomedical research because proteins, not DNA, are the actual mediators of biologic functions within cells as well as of pathophysiology in disease states.

The human genome contains approximately 40,000 genes, whereas the human proteome is estimated to contain more than 1 million proteins.[8] More than 300 posttranslational modifications (PTMs) already have been discovered. Examples include acetylations, carboxylations, and phosphorylations. Each PTM can exist in multiple combinations and various cleaved or spliced forms.[8] Hence, the multidimensionality of proteins compared with that of nucleic acids renders their study much more complicated.

Proteomics encompasses many technical disciplines, including light and electron microscopy, array and chip experiments, genetic read-out experiments such as the yeast two-hybrid assay, and mass spectrometry (MS). Of these various disciplines, MS-based proteomics is the technique of choice for high-throughput analysis of complex protein samples for clinical applications. As our knowledge of the proteins involved in disease pathogenesis expands from mass spectrometric analysis of such complex protein mixtures as serum, urine, and synovial fluid, the protein microarray may become the high-throughput assay that is most efficacious as a diagnostic tool for disease.

Development of MS-based proteomics has been facilitated by several recent advances. Biologic MS evolved in the 1990s as a tool for rapid, powerful large-scale protein analysis, enabling scientists to overcome

the limitations of protein analysis imposed by two-dimensional gel electrophoresis.[9] In addition, major advances in protein ionization with MS techniques have greatly expanded the power of this tool.

MS of individual proteins offers the ability to identify nearly any protein, analyze the protein for the presence of PTMs, characterize its protein-protein interactions, and provide structural information about the specific protein in gas-phase experiments. However, MS of individual proteins does not equate to MS-based proteomics. Proteomics requires a high-throughput simultaneous analysis of many proteins in a specific physiologic state. At present, the advances in proteomics have translated into very few clinically useful applications.

Nevertheless, each technologic breakthrough permits a new type of measurement or improves the quality of data or data analysis, thus expanding the range of potential applications for proteomics research. Our group is using MS-based proteomics to analyze the complex proteins from patients with early and end-stage RA and OA. We hope to identify specific biomarkers and potential new etiologic factors in these diseases.

## Overview of Mass Spectrometry–Based Proteomics

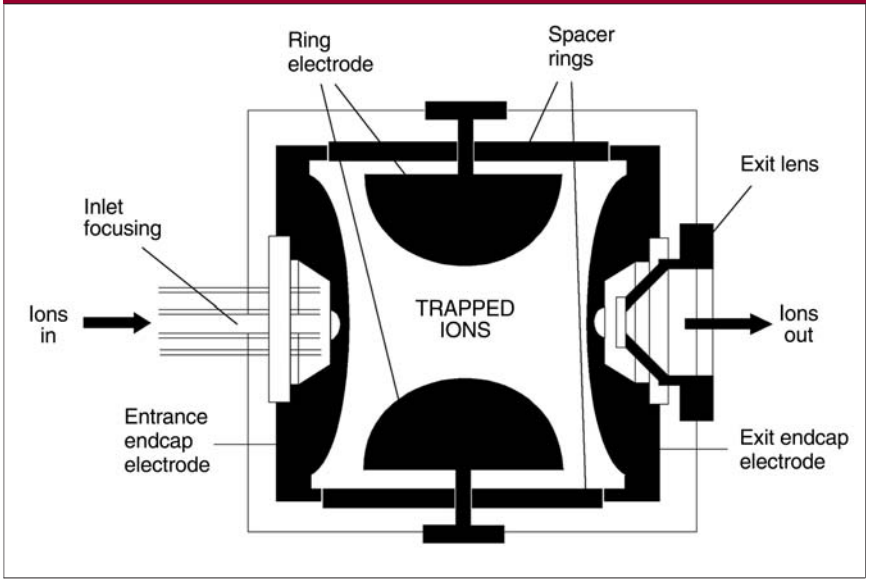
Traditionally, proteins have been identified using one of three techniques: amino acid sequencing using Edman degradation, immunoassays using antibodies for specific epitopes, or MS. These techniques require purified protein and are labor-intensive, low-throughput technologies, especially compared with the contemporary high-speed automated DNA sequencers currently in use for genomics studies, which allow sequencing of 96 bases every 2 hours.

Appreciating the power of MS-based proteomics requires understanding the basic operating mechanism of the mass spectrometer as well as the method of its implementation in proteomics research. The operating principle of all mass spectrometers is based on assignment of an electrical charge to peptide fragments. These fragments are sent through an analyzer under vacuum to detect the mass-to-charge ratio of the peptides.

The two most commonly used techniques to volatize and ionize the proteins or peptides for mass spectrometric analysis are electrospray ionization (ESI), which ionizes the analytes out of a solution, or matrix-assisted laser desorption/ionization (MALDI), which sublimates and ionizes the analytes from a crystalline matrix using laser pulses.[10] ESI-MS is preferred for the analysis of complex mixtures of proteins, whereas MALDI is commonly used for less complex protein mixtures because of its simplicity, excellent mass accuracy, high resolution, and sensitivity. Generally, ESI-based spectrometry is the more efficacious for studying the complex protein mixtures involved in musculoskeletal research.

ESI is normally used in conjunction with an ion trap analyzer, an instrument that "traps" ions for a given time before subjecting them to MS or tandem mass spectrometry (MS/MS) analysis.[11] In proteomics research, one of the most common configurations for ESI on the mass spectrometer is the time of flight (TOF). TOF measures the time of flight of an ion as it traverses a cylindrical tube (ion trap); the longer the time to traverse the tube, the higher the mass of the peptide fragment (Figure 1). Although first-generation three-dimensional ion traps had relatively low mass accuracies, the newer two-dimensional ion traps

**Figure 1**




In mass spectrometers that employ an ion trap analyzer, inlet focusing focuses incoming ions (peptides) within the ion trap. Top and bottom ring electrodes generate a radio frequency in order to isolate specific mass-to-charge ratios. End cap electrodes separate the entering peptides into their constituent amino acids. The exit lens efficiently moves the peptide fragments to the detector within the mass spectrometer. (Reproduced with permission from Dr. Paul Gates, University of Bristol, United Kingdom. Copyright 2004.)

have high sensitivities, mass accuracies, resolution, and dynamic ranges.

## Use of Mass Spectrometry to Generate Protein Identifications

Whole proteins are rarely studied on mass spectrometers because most are too large to ionize effectively. Accordingly, most proteins are first digested by specific proteases (eg, trypsin) into peptide fragments before MS analysis (Figure 2).

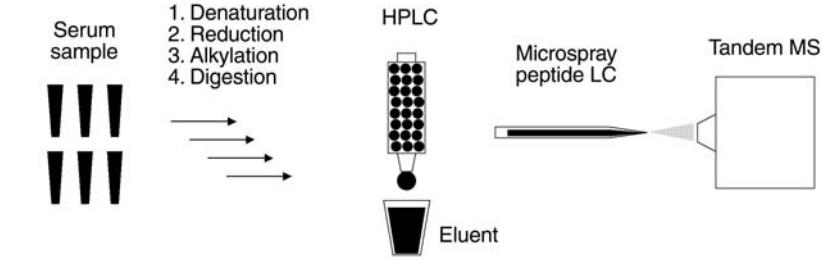
Currently, no technique or instrument exists to both quantify and identify proteins in complex mixtures in a one-step process. Thus, a method of separating mixtures of proteins before analysis on a mass spectrometer is needed. The two most common methods of sample preparation for MS are two-dimensional gel electrophoresis (2DE) and liquid chromatography (Figure 3). In 2DE, proteins are stained, and each protein "spot" is quantified based on the intensity of the stain. These spots are removed from the gel individually and digested with specific proteases before undergoing MS analysis and peptide identification (Figure 4).

Resolution and dynamic range with 2DE are limited in comparison with those achievable with high-pressure liquid chromatography (HPLC). The most popular method for incorporating HPLC in proteomics platforms is two- and three-dimensional chromatographic separations. Two-dimensional chromatographic separations use strong cation exchange and reversed-phase separation; three-dimensional separations employ strong cation exchange, avidin, and reversed-phase separation.

After protein separation, ESI is coupled with ion traps to construct collision-induced dissociation (CID) spectra with the mass spectrometer.[12] A peptide CID spectrum generated from MS analysis can be compared with a comprehensive protein sequence database using various algorithms (Figure 5).

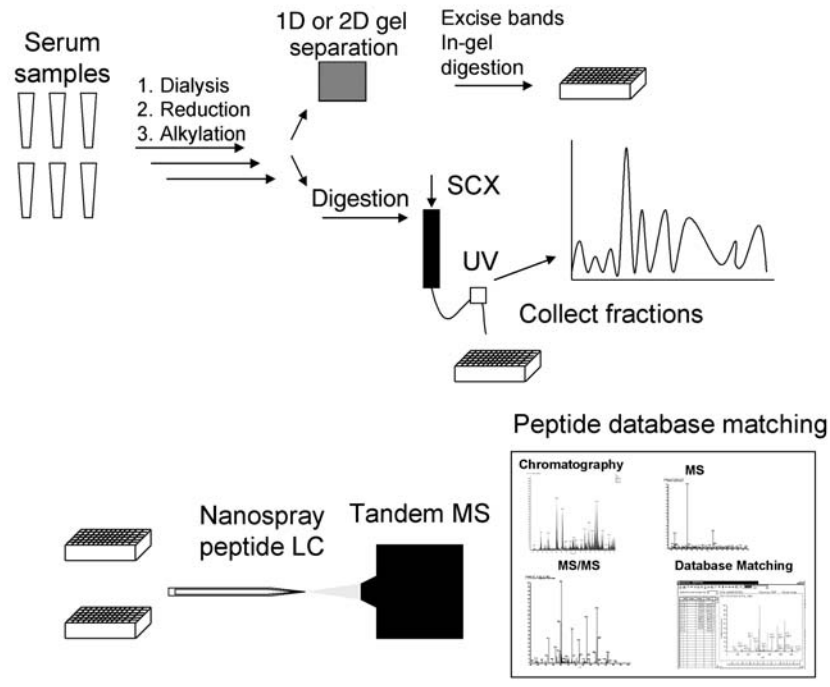
Generally, three methods are used to identify proteins from CID spectra.[10] One method uses peptide sequence tags, which are short peptide sequences specific to a particular protein that are derived from a spectrum's peak pattern. Peptide sequence tags can be used with the

**Figure 2**



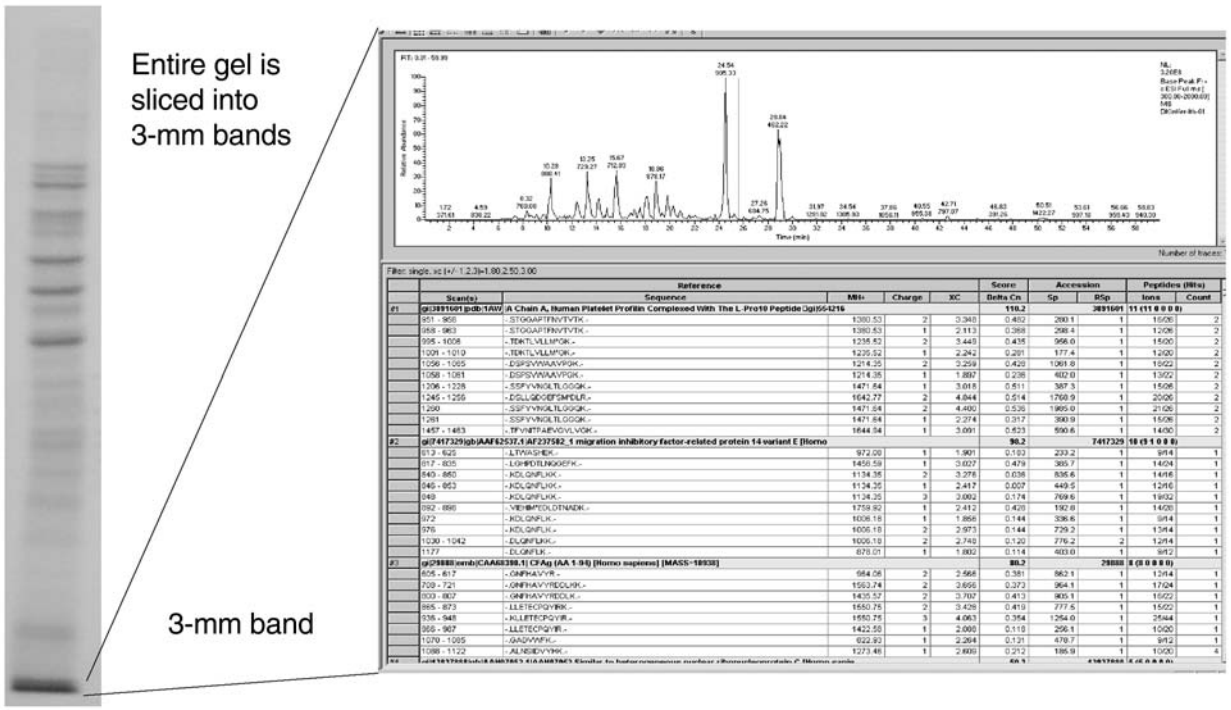

Complex protein mixtures (serum in this example) are first digested with a specific protease, such as trypsin, into peptide fragments before separation on two-dimensional gels or liquid chromatography (LC). The eluent is then analyzed by mass spectrometry (MS). HPLC = high-pressure liquid chromatography

**Figure 3**



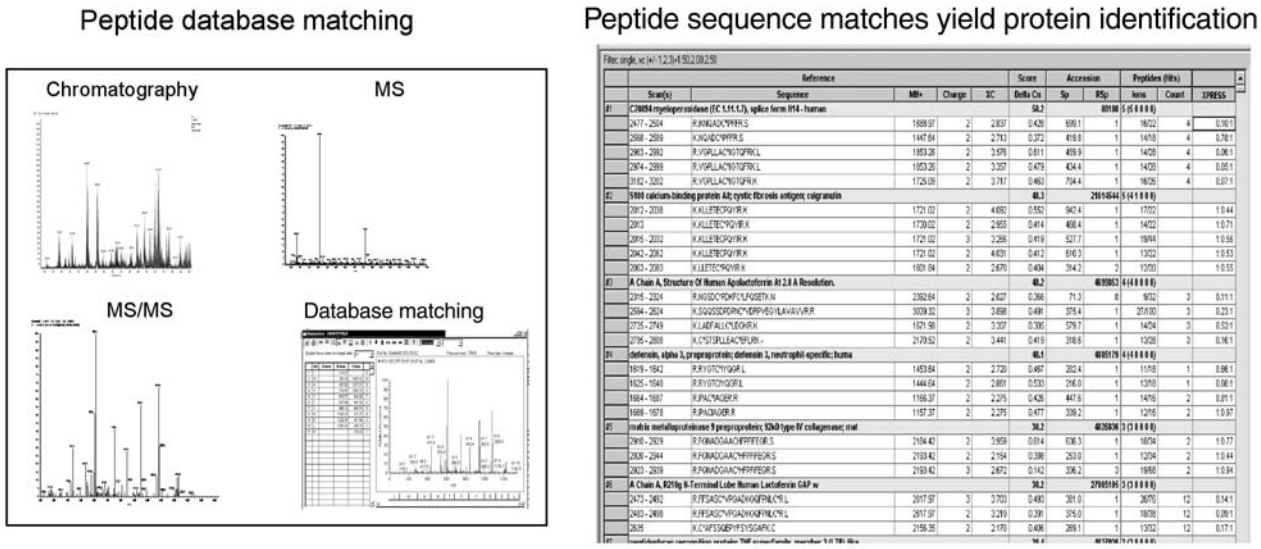

The two most common methods of sample preparation for mass spectrometry: two-dimensional gel electrophoresis (top) and liquid chromatography (bottom). Strong cation exchange separates proteins based on their charge. Ultraviolet laser is used to quantify the amount of peptide within each separated fraction. LC = liquid chromatography, MS = mass spectrometry, SCX = strong cation exchange, UV = ultraviolet laser

**Figure 4**




Gel spots are selectively removed from the gel. The proteins from each band are eluted from the gel and analyzed on the mass spectrometer in tandem. They are then compared to a database of protein sequences to generate probable protein identifications.

**Figure 5**




A peptide collision-induced dissociation spectrum generated from mass spectrometric analysis is compared with a comprehensive protein database using various algorithms to generate protein identifications. MS = mass spectrometry

mass information to determine the "parent" protein. A second method, cross-correlation, uses the theoretic spectra derived from protein databases; a comparative analysis of these spectra with those from the experimental sample yields a matched spectrum and the likely identity of the protein. In the third method, probability-based matching, the calculated fragments from peptide sequences in the database are compared with observed peaks; a score is then generated that correlates to the statistical significance that a given spectrum matches a peptide from the database. Thus, with MS-based proteomics, identification of proteins is limited to species whose proteome has been extensively characterized into protein databases.

## Recent Developments

New methods of combining MS techniques, known as tandem mass spectrometry (MS/MS), have facilitated unprecedented sensitivity and specificity for identifying individual proteins within complex protein mixtures, such as serum or urine. Thus, the goal of determining the proteome of body tissue in specific disease states is becoming a reality.

The development of liquid chromatography–tandem mass spectrometry (LC-MS/MS) is the foundation on which MS-based proteomics is built.[10,13,14] Theoretically, this method of protein analysis can detect very low abundance proteins in a complex mixture of peptides, although significant quantities of protein sample are required and the technique can be tedious. The basic techniques behind LC-MS/MS were pioneered by Hunt et al[13] during their study of major histocompatibility complex class I–associated peptides. Generally, complex protein mixtures are digested with trypsin, usually after preseparation by one-dimensional gel electrophoresis. The peptides are loaded on two- or three-dimensional liquid chromatography columns, and the eluents are analyzed by MS or MS/MS.

MS is a relatively poor instrument for quantification of proteins because of the poorly understood relationship between the measured signal intensity and the quantity of analyte present. As a result, quantitative techniques have been developed for use with LC-MS/MS; the most popular is stable isotope dilution.[15,16] In this method, analytes with the same identity but different stable isotope composition are easily distinguished by MS because of their mass difference. Quantification is achieved using the ratio of signal intensities from the isotopic pairs.

## Protein Microarrays

The generation of profiles of gene expression with DNA arrays has become a powerful tool for studying disease pathogenesis. These arrays have been most effective in delineating the associations between gene expression and specific phenotypes within a particular disease. The most widely researched clinical area using DNA microarray technology is the study of cancers. In a series of studies analyzing breast cancer tissue, for example, DNA microarrays were used to identify differences in gene expression among a series of breast tumor biopsies that allowed for subtyping of these tumors into a basal epithelial-like group, an ErbB2-overexpressing group, and a normal breast-like group.[17,18] A subsequent study was able to demonstrate a difference in outcomes for subjects within each of the subtype cohorts even though patients received the same therapy.[19]

These studies demonstrate the potential usefulness of DNA microarrays in elucidating clinically helpful differences in gene expression among subtypes of specific diseases. However, the inability to detect differences in gene expression represented by proteins directly from biologic fluids is a serious limitation of DNA microarrays. As a result of (1) the lack of a strict linear relationship between DNA expression and the existence of protein end products, (2) the plethora of PTMs intrinsic to most proteins that are not represented by their corresponding DNA sequences, and (3) the inability to directly analyze biologic fluids for biomarkers of disease, the development of protein microarray technology is a major focus in proteomics research.

Protein microarray technology is still in its relative infancy because of the complexity of proteins relative to DNA analysis. One of the key limiting factors for generating protein microarrays with utility for studying specific disease states is the lack of known protein targets for individual diseases. This barrier will likely require more disease-specific data, which will allow a clearer picture of the potential "protein players" involved in specific diseases. Such an insight is likely to result from proteomics studies using MS that deliver high-throughput profiles directly from biologic tissues and that provide the potential protein targets for assimilation onto protein microarrays.

## Proteomics Research Efforts in Osteoarthritis and Rheumatoid Arthritis

Three issues underscore why research into the etiologic mechanisms of OA and RA are ripe for proteomics technology, and for LC-MS/MS in particular. First, the etiologic factors that cause OA or RA remain unknown. Second, proteomics techniques are just starting to be employed in the study of these two disorders. Finally, as a result of limits imposed by preproteomics-era techniques for protein analysis—namely, gel electrophoresis—strategies to identify potential etiologic factors and to determine their protein interactions have focused on hypothesis-driven research. This approach builds on what is already known about a specific disease or mecha-

nism, and it logically investigates plausibly important candidate genes or proteins, one by one. However, the ability to analyze complex mixtures of proteins with high-throughput techniques that permit simultaneous analysis of thousands of proteins has encouraged the development of a discovery-based approach.[20] Still, this discovery-based approach to investigating disease pathogenesis using high-throughput analysis of complex protein mixtures from diseased tissue has not yet been applied to the study of OA or RA.

Currently, RA is diagnosed primarily by criteria from clinical disease manifestations and the presence of rheumatoid factor (IgM-RF) in the serum. Rheumatoid factor is suboptimal because its relatively low specificity and sensitivity limit its diagnostic usefulness in the early phases of disease. Although other autoantigens (including RA33, Sa, p68, calpastatin, perinuclear factor, and antiperinuclear factor) are being studied, none has demonstrated the kind of specificity and sensitivity for RA that translate into a reliable tool for early disease detection.[21-24] The need for a reliable biomarker to detect RA early in the disease is particularly perplexing because most of the contemporary antirheumatic therapies target the disease in its early phases.

Only radiographic and clinical criteria are used to diagnose OA; no biochemical markers for diagnosis have been developed. Thus, diagnosis of OA is usually made clinically once the destruction of articular cartilage is well advanced. Again, the most novel therapeutic interventions, such as cytokine receptor antagonists, are used to stop disease progression in its early stages.

Determination of a protein profile distinct for OA and RA, as well as the identification of candidate proteins involved in the pathogenesis of these diseases, may represent two ideological outcomes from one set of investigations. In other words, the protein profiles determined from an attempt at the complete characterization of the proteome of diseased tissue at various stages of OA and RA may yield proteins that can serve both as potential biomarkers and as plausible candidate proteins for further study. In fact, biomarker acquisition is only a critical first step in a multistep progression to determine the etiologic factors behind OA and RA and, ultimately, to develop therapeutic agents aimed at halting disease progression.

## Current Applications in the Study of Protein Profiles

Although genomics studies have outpaced proteomics applications in the study of OA and RA, early reports on proteomics techniques in arthritis research are surfacing. Ibrahim and Paleolog[25] cite a study by Kato and coauthors on the comparison of protein profiles from serum in patients with RA versus those with OA.[25] In the cited study, 2DE was used to separate the tryptically cleaved peptides derived from normal articular chondrocytes and utilized mass fingerprinting to identify the proteins. Western blotting was then used to detect antigenic protein spots to 20 samples from patients with OA and RA; recombinant fusion proteins with the identified proteins were used to confirm their antigenicity; and enzyme-linked immunosorbent assay was utilized to determine their clinical significance in serum samples from patients with OA and RA. Using this method, four proteins were identified, including human triose phosphate isomerase, as predominantly present in patients with OA. Although there were several limitations to this study, it demonstrates the potential power of proteomics techniques to compare large sets of proteins quickly.

Dasuri et al[26] recently documented their attempt to determine the proteome of fibroblast-like synovial cells derived from patients with late RA using 2DE and MALDI MS. The synovial cells were cultured and subsequently digested before separation with 2DE and MS. The authors were able to identify 254 proteins in fibroblast-like synovial cells, including those implicated as normal physiologic proteins (ie, uridine diphosphoglucose dehydrogenase, galectin 1, and galectin 3) and proteins thought to be potential autoantigens in RA (eg, BiP, colligin, HC gp-39). This study also demonstrates the potential power of proteomics technologies to yield high throughput in a relatively short time.

## Summary

Implementation of proteomics technology may enable identification of protein profiles and potentially new candidate biomarkers and new potential candidate proteins involved in the pathogenesis of both OA and RA. Insights gained from proteomics technology could result in the development of sensitive and specific biomarkers for both OA and RA. These biomarkers would improve our ability to detect these diseases early in their progression and also measure response to treatment. In addition, the novel candidate proteins identified by using these techniques would likely expand our knowledge of disease pathogenesis and yield valuable therapeutic targets for new drug development.

## References

Citation numbers printed in **bold type** indicate references published within the past 5 years.


1. Boskey AL: Musculoskeletal disorders and orthopaedic conditions. *JAMA* 2001;285:619-623.
2. Praemer A, Furner S, Rice DP: *Musculoskeletal Conditions in the United States*. Rosemont, IL: American Academy of Orthopaedic Surgeons, 1999.

3. Schellekens GA, Visser H, de Jong BA, et al: The diagnostic properties of rheumatoid arthritis antibodies recognizing a cyclic citrullinated peptide. *Arthritis Rheum* 2000;43:155-163.
4. Scott DL, Symmons DP, Coulton BL, Popert AJ: Long-term outcome of treating rheumatoid arthritis: Results after 20 years. *Lancet* 1987;1:1108-1111.
5. Pincus T, Callahan LF: Taking mortality in rheumatoid arthritis seriously: Predictive markers, socioeconomic status and comorbidity. *J Rheumatol* 1986;13:841-845.
6. Yelin E, Wanke LA: An assessment of the annual and long-term direct costs of rheumatoid arthritis: The impact of poor function and functional decline. *Arthritis Rheum* 1999;42:1209-1218.
7. Pandey A, Mann M: Proteomics to study genes and genomes. *Nature* 2000;405:837-846.
8. Melton L: Protein arrays: Proteomics in multiplex. *Nature* 2004;429:101-107.
9. Goldring MB: The role of the chondrocyte in osteoarthritis. *Arthritis Rheum* 2000;43:1916-1926.
10. Aebersold R, Mann M: Mass spectrometry-based proteomics. *Nature* 2003;422:198-207.
11. Fenn JB, Mann M, Meng CK, Wong SF, Whitehouse CM: Electrospray ionization for the mass spectrometry of large biomolecules. *Science* 1989; 246:64-71.
12. Aebersold R, Goodlett DR: Mass spectrometry in proteomics. *Chem Rev* 2001;101:269-295.
13. Hunt DF, Henderson RA, Shabanowitz J, et al: Characterization of peptides bound to the class I MHC molecule HLA-A2.1 by mass spectrometry. *Science* 1992;255:1261-1263.
14. Link AJ, Eng J, Schieltz DM, et al: Direct analysis of protein complexes using mass spectrometry. *Nat Biotechnol* 1999;17:676-682.
15. Han DK, Eng J, Zhou H, Aebersold R: Quantitative profiling of differentiation-induced microsomal proteins using isotope-coded affinity tags and mass spectrometry. *Nat Biotechnol* 2001;19:946-951.
16. Gygi SP, Rist B, Griffin TJ, Eng J, Aebersold R: Proteome analysis of low-abundance proteins using multidimensional chromatography and isotope-coded affinity tags. *J Proteome Res* 2002;1:47-54.
17. Hanash S: Disease proteomics. *Nature* 2003;422:226-232.
18. Perou CM, Sorlie T, Eisen MB, et al: Molecular portraits of human breast tumours. *Nature* 2000;406:747-752.
19. Sorlie T, Perou CM, Tibshirani R, et al: Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc Natl Acad Sci U S A* 2001;98: 10869-10874.
20. Hassfeld W, Steiner G, Graninger W, Witzmann G, Schweitzer H, Smolen JS: Autoantibody to the nuclear antigen RA33: A marker for early rheumatoid arthritis. *Br J Rheumatol* 1993; 32:199-203.
21. Despres N, Boire G, Lopez-Longo FJ, Menard HA: The Sa system: A novel antigen-antibody system specific for rheumatoid arthritis. *J Rheumatol* 1994;21:1027-1033.
22. Blass S, Haferkamp C, Specker C, Schwochau M, Schneider M, Schneider EM: Rheumatoid arthritis: Autoreactive T cells recognising a novel 68k autoantigen. *Ann Rheum Dis* 1997;56:317-322.
23. Lackner KJ, Schlosser U, Lang B, Schmitz G: Autoantibodies against human calpastatin in rheumatoid arthritis: Epitope mapping and analysis of patient sera. *Br J Rheumatol* 1998; 37:1164-1171.
24. Nienhuis RL, Mandema E: A new serum factor in patients with rheumatoid arthritis: The antiperinuclear factor. *Ann Rheum Dis* 1964;23: 302-305.
25. Ibrahim S, Paleolog EM: 4th meeting of the EU research network EUROME: From the identification of genes and cellular networks in murine models of arthritis to novel therapeutic intervention strategies in rheumatoid arthritis, London, UK, 9 March 2004. *Arthritis Res Ther* 2004;6:155-158.
26. Dasuri K, Antonovici M, Chen K, et al: The synovial proteome: Analysis of fibroblast-like synoviocytes. *Arthritis Res Ther* 2004;6:R161-R168.