Nutrigenomics and Proteomics in Health and Disease

Food Factors and Gene Interactions
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# Contents

Preface vii  
Contributors ix  

## Section I  Introduction
1. Nutrigenomics and Proteomics in Health and Disease: An Overview  
   Yoshinori Mine, Kazuo Miyashita, and Fereidoon Shahidi  
2. Omics in Nutrition and Health Research  
   Michael Affolter, Frédéric Raymond, and Martin Kussmann  

## Section II  Genomics and Proteomics in Health and Diseases
3. Toward Personalized Nutrition and Medicine: Promises and Challenges  
   Baitang Ning and Jim Kaput  
4. Obesity and Nuclear Receptors: Effective Genomic Strategies in Functional Foods  
   Teruo Kawada, Tsuyoshi Goto, Shizuka Hirai, Rina Yu, and Nobuyuki Takahashi  
5. Inflammatory Genes Involved in Obesity-Induced Inflammatory Responses and Pathologies  
   Rina Yu  
6. Genomics and Proteomics in Allergy  
   Icy D’Silva and Yoshinori Mine  

## Section III  Food Factors–Gene Interactions
7. Beneficial Effects of Conjugated Linoleic Acid  
   Kazunori Koba and Teruyoshi Yanagita  
8. Regulation of Gene Transcription by Fatty Acids  
   Jean-Paul Pégorier  
9. Nonnutrient Functionality of Amino Acids  
   Yoshinori Mine and Connie J. Kim  
10. Functional Bioactive Proteins and Peptides in Nutrigenomics  
    Denise Young and Yoshinori Mine  
11. Antiobesity Effect of Allenic Carotenoid, Fucoxanthin  
    Kazuo Miyashita and Masashi Hosokawa  
12. Control of Systemic Inflammation and Chronic Diseases—The Use of Turmeric and Curcuminoids  
    Stig Bengmark
Contents

13. Alteration in Gene Expression and Proteomic Profiles by Soy Isoflavone 181
   Fazlul H. Sarkar and Yiwei Li

14. Green Tea Polyphenol-Modulated Genome Functions for Protective Health Benefits 201
   Molay K. Roy, Mahendra P. Kapoor, and Lekh R. Juneja

15. Oat Avenanthramides: A Novel Antioxidant 239
   Li Li Ji, Ryan Koenig, and Mitchell L. Wise

16. Cancer-Preventive Effects and Molecular Actions of Anthocyanins 251
   De-Xing Hou

17. Food Components Activating Capsaicin Receptor TRPV1 263
   Tatsuo Watanabe, Yasaku Iwasaki, Akihito Morita, and Kenji Kobata

   Takanori Tsuda and Hitoshi Matsumoto

19. Licorice Flavonoids 291
   Shinichi Honda, Yuji Tominaga, and Shinichi Yokota

20. Isoprenols 301
   Tsuyoshi Goto, Nobuyuki Takahashi, Shizuka Hirai, and Teruo Kawada

21. Anti-inflammatory and Anticarcinogenesis Potentials of Citrus Coumarins and Polymethylated Flavonoids 311
   Akira Murakami and Hajime Ohigashi

22. Probiotics: Food for Thought 325
   Kingsley C. Anukam and Gregor Reid

Section IV Advanced Analytical Techniques for Nutrigenomics and Proteomics

23. Microarrays: A Powerful Tool for Studying the Functions of Food and Its Nutrients 339
   Hiroshi Mano, Jun Shimizu, and Masahiro Wada

24. Challenges and Current Solutions in Proteomic Sample Preparations 351
   Feng Tao

25. Computational Methods in Cancer Gene Networking 367
   Edwin Wang

26. Peptidomics 375
   Yoshinori Mine

Index 387
Preface

Recent advances in the areas of functional foods, nutraceuticals, and natural health products have been culminated by those in modern molecular nutrition. Thus, the advent of nutrigenomic, proteomic, and metabolomic has resulted in a leap toward individualized nutrition, hopefully in the near future. In this connection, the present book on nutrigenomic and proteomic is expected to provide links and information relevant to health promotion and disease risk reduction. Many of the bioactive components present in foods or produced upon ingestion or upon processing under conditions mincing digestion are found to improve health status related to cardiovascular diseases, certain types of cancer, inflammatory disorders and immune response, diabetes, gastrointestinal tract conditions as well as various psychological problems, and the metabolic syndrome. The techniques used to study such benefits have improved over the recent years and unique tools have now become available that facilitate undertaking of challenges thought impossible only a decade ago.

This book provides a state-of-the-art compilation of the most recent developments in the exciting field of nutrigenomics and proteomics. It is of special interest to nutritionists, food scientists, biochemists, pharmacologists and biologists, among others. This book serves as a reference compendium for scientists in academia, industry, and government laboratories. It may also be used as a text for senior undergraduate and graduate students in multidisciplinary areas listed. We are indebted to world-renowned scientists for their excellent contributions that made the publication of this book possible.

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Section I
Introduction
Nutrigenomics and Proteomics in Health and Disease: An Overview

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Association between diet and chronic diseases has long been recognized through epidemiological studies. Modern molecular nutrition focuses on health promotion, disease risk reduction, and performance improvement through diet and lifestyle considerations (Kussmann and Blum 2007; Ronteltap et al. 2008). New genomic, proteomic, and metabolomic techniques are now enabling us to find out more about the basis of these associations through examination of the functional interactions of food with the genome at the molecular, cellular, and systemic levels (Corthésy-Theulaz et al. 2005; Kato 2008; Mariman 2006). The human genome is estimated to encode over 30,000 genes and to be responsible for generating more than 100,000 functionally distinct proteins. While traditional nutrition research has dealt with providing nutrients to nourish populations, nowadays it focuses on improving health of individuals through diet. Modern nutritional research is aiming at health promotion and disease risk reduction and on performance improvement (Trujillo et al. 2006). Nutrigenetics questions as to how individual genetic disposition, manifesting as single nucleotide polymorphisms, copy-number polymorphisms, and epigenetic phenomena, affects susceptibility to diet. Nutrigenomics addresses the inverse relationship; that is, how diet influences gene transcription, protein expression, and metabolism. Metabolomics is a diagnostic tool for metabolic classification of individuals. A major methodological challenge and first prerequisite of nutrigenomics is integrating genomics (gene analysis), transcriptomics (gene expression analysis), proteomics (protein expression analysis), and metabolomics (metabolite profiling) to define a “healthy” phenotype (Kussmann et al. 2006; Milner 2004). The long-term deliverable of nutrigenomics is personalized nutrition for maintenance of individual health and prevention of disease (Fay et al. 2008; Kaput 2008; Ronteltap et al. 2008). “Nutrigenomics” may offer a new approach for understanding the beneficial effects of dietary compounds on the development of severe polygenic diseases, such as cardiovascular disease, diabetes, and hypertension (Keusch 2006).


Chapter 1 summarizes aims and scope as well as overall highlights of this book. Chapter 2 consists of introductory omics in nutrition and health research. Nutrigenomics contains the three omics disciplines gene, protein, and metabolite profiling (transcriptomics, proteomics, and metabolomics) as applied to the field of nutrition and health. Furthermore, nutrigenomics forms the scientific basis for developing nutrition adapted to the specific needs of (rather large) consumer groups, be they healthy, at risk, or diseased. The three omics platforms are introduced in this chapter that also describes their application in nutritional research. Microarray-based gene expression analysis is the most mature genome-wide profiling platform. Consequently, transcriptomics in nutritional studies is widely applied when it comes to basic and preclinical research in either cell culture systems or animal models. Proteomics has evolved as an analog to genomics, from identifying all proteins present in a given sample at a given time to a global molecular analysis platform addressing functional aspects of biological systems. Comparing such variations in the proteome enables the discovery of key proteins and
the identification of modulated pathways involved, for example, in specific nutrition-related processes. Over the last two decades, proteomics has developed into an established technology for biomarker discovery, clinical applications, disease profiling and diagnostics, and the study of protein interactions and of the dynamics of signaling pathways. Metabolites represent the endpoints of metabolism and can provide information on the molecular events associated with the adaptations of the body to increased or decreased fluxes of nutrients through metabolic pathways. Metabolomics in nutrition addresses the challenge of characterizing food-related metabolic modulations. Moreover, individual metabolites such as cholesterol, glucose, and homocysteine are considered as markers for health or disease status. Nutrigenomics and nutrigenetics are key science platforms to promote health and prevent disease through nutrition that better meets the requirements and constraints of consumer groups with specific health conditions and particular lifestyles. Section II comprises three chapters on the impact of nutrigenomic and proteomic interventions on health and diseases. Chapter 3 deals with personalized nutrition and medicine. The concept of personalizing nutrition and medicine—and therefore healthcare—emerged from the human genome and haplotype projects. The results of these large-scale, international initiatives offered the hope that nutrition and medicine could be tailored to the individual. The significant advances in understanding complex biological processes relied on reductionistic approaches: hold all variables but one constant. While this strategy was successful for certain monogenic phenotypes, understanding complex systems requires analytical approaches that incorporate rather than avoid complexity. The key challenge for personalizing healthcare then is not the complexity of the data sets, but acquiring those data sets in a manner to reduce noise and increase true signals. This might best be accomplished by preselecting phenotypes based on quantitative data, or alternatively, preselecting genotypes that maximize differences in allele frequencies of candidate genes involved in nutrient metabolism or other physiological traits. The integrative whole system analyses of the data sets and new visualization methods such as shown with network analysis tools provide a path not only to perform these complex experiments, but also to develop biological insight into the outcomes. The development of nutrigenomics and genetics and the application of this knowledge will provide strategies for maintaining health and improving medical treatment of chronic diseases.

Chapters 4 and 5 discuss obesity and nuclear receptors and inflammatory genes involved in obesity-induced inflammatory responses and pathologies. Obesity is the state of excessive formation of adipose tissues. Recent research has clarified the differentiation of adipocytes, the level of subsequent fat accumulation, and the secretion of the biologically active adipocytokines by adipocytes. In particular, it has been clarified that adipocytokines secreted by adipocytes play a significant role in the pathogenesis of diseases such as diabetes and cardiovascular diseases and are closely associated with the pathogenesis and exacerbation of ailments arising from obesity. This chapter discusses obesity and the metabolic syndrome and then describes the nuclear receptors that are most important in adipocyte differentiation and the mechanism underlying the expression of function of adipocytes affecting obesity from the viewpoint of nutrigenomics. Obesity is also a low-grade systemic chronic inflammatory condition, characterized by abnormal cytokine production, increased acute phase proteins, and other inflammatory mediators. Obesity-induced inflammation consists of a set of inflammatory immune components and inflammatory signaling pathways similar to those involved in classical inflammation, such as inflammatory cells like macrophages, inflammatory mediators like cytokines and chemokines, as well as inflammatory signaling molecules. Obesity-induced inflammation is considered to serve as the potential mechanism linking obesity to obesity-related pathologies such as insulin resistance, type 2 diabetes, fatty liver disease, atherosclerosis, some immune disorders, and several types of cancer. Chapter 5 specifically focuses on obesity-induced inflammatory components, linking to obesity-related pathologies. Adipose tissue-derived inflammatory genes/proteins such as adipocytokines and signaling molecules and the inflammatory cross-talk within adipose tissue cells through adipocytokine. Allergies affect almost 20% of the population in the developed world and allergies can be life-threatening. Individuals may be allergic to a variety of natural or synthetic molecules, such as foods, drugs, chemicals, dust, pollen, and metals. Genomic and proteomic methods are powerful techniques for the identification, characterization, and in vitro diagnosis of allergies. Chapter 6 describes molecular mechanisms of allergy and gene interactions and susceptibility to allergic responses. It also reports on recent therapeutic approaches for allergies using recombinant DNA techniques.

Section II includes various food factors–gene interactions and their impact in health and diseases. This section consists of 16 chapters that cover lipids, proteins/peptides/amino acids, cartenoids, phytochemicals, and probiotics. Chapters 7 and 8 deal with the beneficial effects of conjugated linoleic
acid (CLA) and regulation of gene transcription by fatty acids. CLA has been shown to exert various physiological functions, other than antimutagenicity, such as anticarcinogenic and antiobesity (reduction of body fat mass) activities, prevention of atherosclerosis, enhancement of immune function, and suppression of blood pressure, despite the fact that physiological properties of CLA are still limited. The physiological effects of CLA are also described along with potential health benefits of conjugated linolenic acid. Dietary fat is an important macronutrient required for the growth and development of all organisms. Excessive levels of dietary fat or imbalance in its composition (saturated versus unsaturated fat) have been related to the onset or development of several chronic diseases such as coronary artery disease, obesity, and type 2 diabetes as well as certain types of cancer. The biological functions of lipids are mainly carried out by fatty acids and/or derived signaling molecules such as ceramides, diacylglycerols, eicosanoids, and coenzyme A thioesters (acyl-CoA). The last two decades have provided evidence that major (glucose, fatty acids, amino acids) or minor (iron, vitamin, etc.) dietary constituents regulate gene expression in a hormone-independent manner. The molecular mechanisms by which fatty acids and/or their metabolites control the transcription of genes involved in their own metabolism or in carbohydrate metabolism are also described. These effects are mediated either by direct binding on transcription factors such as PPARs, LXR, HNF-4, RXR, etc. (each belonging to the nuclear receptor superfamily) or alternatively through modifications in nuclear abundance and/or activity of numerous transcription factors such as SREBP-1c, ChREBP, and NF-κB.

Chapter 9 focuses on amino acid biological functions as nonnutrient. Although amino acids are widely known as the building blocks of proteins, their functions in living organisms are vast as they can interact with the endocrine, neuronal, and immune systems to influence the balance between health and disease. These systems, particularly in diseased states, affect the amino acid availability and may induce pathways to alter protein synthesis. The underlying mechanism of the regulation of the biological functions is partially due to amino acid control of gene expression. This chapter reviews the importance of amino acid balance and the consequences of amino acid imbalance at the genetic level. Health and disease implications through amino acid deficiency and supplementation was explored. Many amino acid studies have reported health benefits during diseased states, such as cancer, inflammatory disorders, diabetes, gastrointestinal disorders, and muscular wasting diseases. Understanding the mechanism of amino acid control of genes, both singly and in unison, may provide its involvement in disease progression and prevention. Many researchers have reported that food proteins and their peptides express a variety of functions in the body, including a reduction of blood pressure, antimicrobial activity, antioxidative, anti-inflammatory, antisatiety, anticancer, antiobesity, antiallergy, modulation of immune cell functions, and regulation of nerve functions. Bioactive peptides are peptide sequences present in the intact protein that under normal circumstances do not have biological properties, but when they are released as peptides in vitro or in vivo, they exert biological activities. There is increasing commercial interest in the production of bioactive peptides from various sources such as egg, milk, cereal, and fish proteins. Chapter 10 summarizes recent advances of food-derived bioactive peptides–gene interactions and their mechanisms of actions. Although their properties and physiological effects have not been completely explored, bioactive peptides can broadly be divided into two categories: (1) peptides that exert their effects by direct physical interaction with another molecule, and (2) peptides that interfere with gene expression. Bioactive peptides that alter gene expression can do so by (1) epigenetic modification of the proteins that attach to the DNA, (2) alteration of the cell’s primary signaling ligand to indirectly influence transcription factor activity, and (3) interference with cell signaling and gene expression via direct binding of peptide ligand to receptor. Understanding the behavior of dietary proteins and peptides in the intestine is also important for designing functional foods with physiological functions.

Carotenoids represent a large group of isoprenoid structures with many different structural characteristics and biological activities. To date, a wide range of carotenoids have been isolated, identified, and quantified from the extracts of fruits and vegetables commonly consumed in the world. The best known biological function of carotenoids is their established role as pro-vitamin A. Chapter 11 describes the nutrigenomic study on the anti-obesity effect of allelic carotenoids from seaweeds and vegetables, with special reference to their regulations on relative gene and protein expressions. Fucoxanthin and neoxanthin are the major carotenoids present in chloroplasts of brown seaweeds and higher plants, respectively. Fucoxanthin is the most abundant of all carotenoids, accounting for >10% of the estimated total natural production of carotenoids. The key for success of fucoxanthin will be induction of uncoupling protein 1 in white adipose tissue (WAT) and downregulation of adipokines such as TNFα. The regulatory effect of fucoxanthin on PPARγ and γ3-AR in WAT is
correlated with its antiobesity and antidiabetic effects. Furthermore, the relationship between carotenoid structure and suppressive effect on the differentiation of 3T3-L1 adipose cells shows that carotenoids containing an allene bond and an additional hydroxyl substituent on the side group may show the characteristic antiobesity activity.

Chapter 12 deals with the control of systemic inflammation and chronic diseases by the use of turmeric and curcumenoids. Numerous plant-derived, but also microbially derived, substances, often referred to as chemopreventive agents, have documented anti-inflammatory effects and are believed to reduce the rate of aging and prevent degenerative malfunctions of organs and also development of acute and chronic diseases. Among these are various curcumenoids, the active ingredients in turmeric and curry-containing foods, and thousands more of hitherto little or totally unexplored substances. This chapter focuses on documented experimental and clinical effects of supplementation of turmeric, various curcumenoids, and pure curcumin. The Food and Drug Administration (FDA) has approved a health claim for soy-based food products for health benefits primarily based on epidemiological data indicating that high soy consumption is associated with a lower risk of cardiovascular diseases. Soy isoflavones also show a beneficial role in obesity, diabetes, coronary artery disease, and osteoporosis in postmenopausal women. Soy isoflavones have been shown to inhibit carcinogenesis and cancer cell growth in vivo and in vitro. It has also been found that soy isoflavones lower total cholesterol and low-density lipoprotein cholesterol, suggesting the effect of isoflavones on cardiovascular disease risk reduction. Chapter 13 presents gene expression and proteomic profiling by soy isoflavones. It has been found that soy isoflavones regulate the expression of genes that are related to estrogen regulation, organ differentiation, and fat and bone metabolism in normal cells. Soy isoflavones also inhibit the growth of cancer cells through the modulation of genes, which control cell proliferation, cell cycle, apoptosis, oncogenesis, transcription regulation, and cell signal transduction system. In this chapter, current evidence on the molecular effects of soy isoflavones as documented by nutrigenomic and nutriproteomic research is provided.

Over the last two decades more than five thousand peer-reviewed articles and tens of thousands of news articles have provided evidence for enhanced health benefits of tea consumption. At present, multiple evidences have proven the involvement of tea beverages in health promotion that are directly linked to its polyphenol content. Green tea has firmly established its powerful strength in reducing oxidative stress, suppressing cancer-related risks, cardiovascular disease, neuronal damage, and hepatic disorders, among others. Epidemiological and clinical studies have also proven that individuals consuming tea or many form of tea polyphenols benefit from a lower incidence of cancers and other lifestyle-related diseases such as diabetes, obesity, and cardiovascular disease, among others. However, the question that is duly continued to be answered is how green tea polyphenols exert their health beneficial effect? Chapter 14 explores how green tea polyphenols modulate genome functions for protective health benefits. Is it a simple site-specific activity or alteration of a pathway that ultimately lead to altered activity of one or more secondary molecules required to maintain normal cell function or enhancing the meaningful roles of the molecules to maintain cell machinery systems? This chapter reviews how green tea polyphenols modulate genome function, gene repair, protecting genes, and exerting the roles considered auspicious, that even remained unknown until a decade ago. This chapter also lists the latest evidences in accordance with the enhanced philofunctional functions. Reactive oxygen species are generated ubiquitously in aerobic organisms. When these cytotoxic agents overwhelm endogenous antioxidant defense systems, serious oxidative stress and damage occur as reflected by the oxidative modification of macromolecules such as lipid, protein, and DNA. Thus, it is critical that cells maintain optimal antioxidant defenses in order to reduce oxidative damage. Dietary supplementation and therapeutic use of antioxidants are emerging measures to prevent and treat oxidative stress-induced diseases. Chapter 15 describes oat avenanthramides as novel antioxidants. Oat (Avena sativa), although consumed in considerably lower quantities worldwide than wheat and rice, has a highly edible quality and contains high nutritional value compared to other minor grains. Over the past decade, interest of restoring oat as a natural antioxidant additive in food has been on the rise. Other than tocopherols, tocotrienols, and flavonoids, oat contains a unique group of approximately 40 different types of polyphenolic compounds called avenanthramides (AVA) that consist of an anthranilic acid derivative and a hydroxycinnamic acid derivative linked by an amide bond similar to those found in peptides. There is strong evidence that AVA are potent inhibitors of cell proliferation and inflammatory processes, especially in the endothelial cells and smooth muscle cells of blood vessels. These effects have been shown to be mediated by its inhibition of proinflammatory cytokine production and signaling. AVA have also been reported
to modulate endogenous antioxidant defense such as increasing plasma glutathione level and upregulating tissue superoxide dismutase activity, the mechanisms of which remain to be elucidated. Chapter 16 reviews cancer-preventive effects and molecular actions of anthocyanins. Anthocyanins are naturally occurring polyphenolic compounds that confer an intense color to many fruits and vegetables. A few population-based investigations have highlighted the potency of anthocyanins or anthocyanin-containing mixtures on cancer prevention or cancer risk reduction. Studies on animal models have revealed that high intake of anthocyanins or anthocyanin-containing mixtures protects against tumorigenesis of colon, skin, and mammary glands. Extensive studies in cancer cell lines have shown the inhibitory effects of anthocyanins or anthocyanin-containing mixtures on the growth of cancer cells derived from malignant human tissues including vulva, stomach, colon, lung, breast, leukemia, uterus, mouth, and prostate. Recent molecular data have demonstrated that anthocyanins could modulate oncogenic cellular signaling transduction pathways (MAPK and EGFR), transcriptional factor activations (AP-1, NF-κB, p53), and downstream gene expressions (COX-2, iNOS, Bax). These molecular actions are involved in the processes of cell transformation, inflammation, and apoptosis, which provide molecular basis for the cancer-preventive effects of anthocyanins.

Chapter 17 deals with how food components activate capsaicin receptor, transient receptor potential vanilloid subtype 1 (TRPV1). Capsaicin is a pungent principle of hot pepper. Capsaicin exerts several biological activities such as causing burning sensation, stimulating primary afferent neurons conducting chemical pain or hotness, enhancing energy metabolism, showing protection against stomach mucosa, inducing apoptosis in some cancer cells, and so on. Many of them are exerted through capsaicin receptor activation. Because obesity is one of the serious factors on lifestyle-related diseases such as hypertension, stroke, diabetes, and hyperlipemia, this chapter focuses on the thermogenic action or body fat lowering effect of capsaicin. Thermogenic action of capsaicin is thought to be exhibited through activation of TRPV1. From the discovery of TRPV1 gene in 1997, food components activating TRPV1 have been vigorously investigated. There are lists of capsaicinoids of hot pepper, piperine of black pepper, eugenol of clove, ginsenosides of Asian ginseng, and evodiamine of Evodia rutaecarpa, among others. Capsiate inhibits accumulation of body fat in humans. Anthocyanins are the largest group of water-soluble pigments in the plant kingdom. In the human diet, they are derived primarily from a wide variety of plant sources including crops, beans, fruits, vegetables, and red wine, and their effects are also diverse and important to health promotion. Chapter 18 focuses on blackcurrant (Ribes nigrum L.) anthocyanins because blackcurrant is rich in it and blackcurrant is consumed in many countries. This chapter provides a review of the newly discovered effects of anthocyanins including their antiobesity effect, antidiabetes effect, and vision improvement. Chapter 19 describes various biological activities of licorice. Licorice, the root of the leguminous Glycyrrhiza plant species, is one of the most useful and popular plants in both Asia and Europe, and the history of its consumption as a traditional medicine and food goes back to over 4,000 years to the era of ancient Mesopotamia and Egypt. Licorice contains triterpenes and phenolic constituents such as glycyrrhizin, a well-known typical active constituent of licorice, and the species-specific constituents glabridin, glycycoumarin, and licochalcone A in G. glabra, G. uralensis, and G. inflato, respectively. In G. glabra, the species specific compound is glabridin. Various studies have shown the biological effects of glabridin, licorice, or its extracts. These include antioxidative, estrogen-like, anti-inflammatory and anti-Helicobacter pylori activities. Hydrophobic flavonoids from G. glabra are extracted and concentrated, and the resulting extract is referred to as licorice flavonoid oil (LFO). DNA microarray analysis suggests that the antiobesity effects of LFO are attributable to suppressed fatty acid synthesis and activated fatty acid catabolism in the liver. LFO has also received FDA approval as a new dietary ingredient in the United States in 2006. Therefore, further studies that elucidate the mechanism of LFO containing licorice hydrophobic flavonoids would contribute to the efficient application of LFO in the treatment of metabolic syndrome. Isopentyl diphasphate and its isomer dimethylallyl diphasphate are the universal five-carbon precursors of isoprenoids. Isoprenoids are contained in many herbal plants, and several isoprenoids have been shown to be available for pharmaceuticals, for example, artemisinin and taxol as malaria and cancer medicines, respectively. Various isoprenoids are contained in many plants not only for herbal use but also for dietary consumption. Chapter 20 reports on several bioactive isoprenoids, contained in herbal or dietary plants, which have possibilities to ameliorate metabolic disorders via activation of ligand-dependent transcription factors, that is, nuclear receptors. Chapter 21 reviews anti-inflammatory and anticarcinogenic potential of citrus coumarins and
polymethylated flavonoids. Citrus fruits are well known to contain an array of secondary metabolites in terms of their chemical structures and biological activities, which biosynthesize monoterpenes (d-limonene, etc.), triterpenes (limonoids), flavonoids (nobiletin, hesperidin, etc.), coumarins (auraptene, bergamottin, etc.), and carotenoids (β-carotene, β-cryptoxanthin, etc.). Ample evidence obtained from in vitro and in vivo experiments as well as epidemiological surveys indicates that frequent intake of citrus fruits is beneficial to human health. These citrus compounds are hydrophobic and thus tend to localize in gastrointestinal mucosa in rodents as compared to general polyphenols present. Thus, abundant data have revealed both auraptene and nobiletin to be highly promising citrus components with anti-inflammatory and anticancer activities, with notable action mechanisms and effects on metabolism. One of the distinct characteristics of citrus fruits, as compared with other foods, is the variety of active constituents in terms of chemical characteristics and bioactivities. Thus, combination studies using different types of citrus components for enhancing each efficacy are warranted, such as combining nobiletin (targeting COX-2 transcription) and auraptene (targeting COX-2 translation) to determine their additive or even synergistic effects.

Food and Agricultural Organization of the United Nations and the World Health Organization define probiotics as “live microorganisms which when administered in adequate amounts confer a health benefit on the host.” The majority of probiotics are strains of lactobacilli or bifidobacteria and they are administered in food products such as yogurt, milk drinks, and cheese, as well as capsules and tablets. The effect that beneficial microbes have on health maintenance is becoming more and more recognized, given the realization that so many organisms reside in the human body. The reintroduction of beneficial organisms (probiotics) to the host has mostly been via food and dietary supplement products, and thus relevant to nutrigenomics. Chapter 22 discusses some examples of how probiotic microbes and their proteinaceous and other by-products contribute to health. As more human and microbial genomic information emerges, it will become clearer under what conditions probiotic organisms interface with the host in an optimal way.

Section IV highlights recent advances in analytical techniques for nutrigenomic and proteomic research in food and health. Chapter 23 describes microarray as a powerful tool for studying the functions of food and its nutrients. Microarray is a high-throughput genomic tool. It can be used for profiling and monitoring the expression levels of tens and thousands of genes (entire genomes). It can also be used to determine the influence of food nutrients and/or bioactive compounds (food factors) on metabolic pathways and to understand how food nutrients and factors maintain homeostatic control of gene expression levels. Microarray technology is a “nutrigenomics” tool and can be used to investigate the levels of transcripts in particular. Typically, food is a complex and variable mixture of nutrients and other components. Most food factors are weak dietary signals and must be considered in the context of chronic exposure. Microarray analysis clearly indicates the effects exerted by food factors and nutrients on metabolic pathways via transcriptome modifications. Moreover, the results of microarray analysis suggest that food factors and nutrients influence the metabolome because alterations in the transcriptome cause changes in the metabolome. Therefore, microarray analysis is one of the most convenient tools for inferring the proteome and metabolome. This technology will enhance understanding of the manner in which food and nutrition influence metabolic pathways and how these factors maintain homeostasis under normal conditions or diet-related or non-diet-related disease conditions. Chapter 24 highlights challenges and current solutions in proteomic sample preparation. Proteomics is a discipline of relatively short history, but it holds great promise in elucidating biochemical information via quantitative determinations of the whole collection or representative proteins. One of the common objectives in proteomic studies is the discovery of biomarkers. Although biological systems are extremely complex, and the technology challenges are still many, hundreds and thousands of biomarker candidates are being discovered with advancements made in proteomic technologies. One of the major hurdles in proteomics is the identification of true biomarkers via analytical and clinical validation studies. This chapter reviews some critical aspects of biomarker determination using proteomic methods and some examples of new developments in the proteomic sample preparation techniques, particularly the “pressure cycling technology.” In the past few years, many high-throughput techniques have been developed and applied in biological studies. These techniques such as “next generation” genome sequencing, chip-on-chip, and microarray, among others, can be used to measure gene expression and gene regulatory elements in a genome-wide scale. Moreover, as these technologies become more affordable and accessible, they have become a driving force in modern biology. Traditionally, biologists described these relationships between a limited number
of genes or proteins using a descriptive language. With the huge amount of data produced by high-throughput techniques, biologists have to deal with thousands of biological relations in a single experiment. In this situation, the traditionally descriptive ways for biological relations are not sufficient to deal with the huge number of relations under study. The only way to deal with a large amount of relations is through mathematical representations and computations by researchers in biological sciences. Chapter 25 first introduces basic computational concepts and then illustrates the procedures and computational techniques for high-throughput data analysis, using examples from cancer research. Proteomics is central to nutrigenomics and has the potential to explain many of the physiological changes associated with nutritional stimuli. In proteomics, all proteins expressed in a cell or tissue are analyzed to identify the presence or absence of some key proteins that provide information about the early stages of disease or different conditions. However, a comprehensive analysis of peptides and small proteins of a biological system corresponding to the respective genomic information was missing in proteomics. Chapter 26 introduces the concept of peptidomics. The term peptidomics was first introduced as a subset of proteomics for the description of peptides as gene products in February 2000 at the ABRF conference “From Singular to Global Analysis of Biological Systems.” This was coined as a short version of “peptide proteomics” and was defined as the technology for comprehensive qualitative and quantitative description of peptides in a biological sample. Studies of peptidomics cover peptides with low-molecular-weight and small proteins (0.5–15 kDa), since peptides among the families of hormones, cytokines, and growth factors play a central role in many physiological processes. In addition, application of peptidomics knowledge to the nutrient effect may yield potential information about the diet-induced peptide changes and may act as good biomarkers. However, the field of peptidomics is relatively new and has potential to progress in future with the advent of high-throughput mass spectrometry-based technologies coupled with bioinformatics and genomic databases.

Completion of human genome project coupled with the advancement in “omic” technologies enabling researchers to analyze the complex interplay of metabolism, gene expression, and function, and more broadly, genetic diversity within and between human populations. Nutrition science has broadened to the new discipline of nutrigenomics, which allows an in-depth understanding of metabolism, health, and pathophysiology of disease that ultimately could be used to prevent or treat diseases. The major goal of this book is to comprehensively understand the response of the body’s genes to diets and food factors through various omics technologies such as transcriptomics, proteomics, and metabolomics. This will contribute to the development of new preventive and therapeutic strategies for both pharmacological and nutritional interventions (Bauer et al. 2004; Mariman 2006; Milner 2007).

The editors have succeeded in bringing together many renowned international experts in nutrigenomics and proteomics in health and diseases. We are grateful to all the authors for their state-of-the-art compilation of recent rapid development in this field. We believe that this book certainly deserve a broad readership in the disciplines of nutrition, pharmacology, nutraceutical/functional foods, food science, biology, biochemistry, biotechnology, and life science. This book could also be used as a reference book by senior undergraduate and graduate students as well as nutraceutical and pharmaceutical industry.

REFERENCES


INTRODUCTION

Nutrients and genomes interact. Nutrition is the most important lifelong environmental impact on human health status. While nutrigenetics addresses how an individual’s genetic makeup predisposes for susceptibility for dietary intake, nutrigenomics rather asks how nutrition influences the expression of a given genome.

Nutrigenomics contains the three omics disciplines—gene, protein, and metabolite profiling (transcriptomics, proteomics, and metabolomics)—as applied to the field of nutrition and health. Together, they are a prerequisite for nutritional systems biology; that is, the understanding of the dynamic interaction between food components and the entire diet with cells, organs, and the whole body. Nutrigenomics furthermore forms the scientific basis for developing nutrition adapted to the specific needs of (rather large) consumer groups, be they healthy, at risk, or diseased. This chapter introduces the three omics platforms and describes their application in nutritional research. We also discuss current limitations, recommend future developments, and highlight the opportunities for omics integration and correlation with genetics in a nutritional context.

TRANSSCRIPTOMICS IN NUTRITION AND HEALTH RESEARCH

Microarray-based gene expression analysis is the most mature genome-wide profiling platform. Consequently, transcriptomics in nutritional studies is widely applied when it comes to basic and preclinical research in either cell culture systems or animal models. The mRNA profiling bears the potential to identify specific transcript changes as a response to the administration of a nutrient or non-nutrient compound, or to a treatment or dietary intervention in a well-defined experimental setting. The observed changes in mRNA level are not necessarily causal markers; they might rather represent a pattern of expressed transcripts that changes in a characteristic and reproducible way. Gene expression profiling has the character of a screening process covering thousands of potential indicators of the (changed) metabolic status and, therefore, it often also reveals unexpected findings.

MICROARRAY-BASED GENE EXPRESSION PROFILING

Although microarrays are not the only available technology for genome-wide gene expression profiling, it has established itself by far as the most widely deployed in research. This is mainly due to a range of commercially available platforms and the meanwhile high level of standardization. Today’s microarray platforms are based on either single long or multiple short oligonucleotides as probes. They have different manufacturing procedures and use different labeling methods. The arrays display probes that hybridize with high sensitivity and specificity with their counterparts from the sample. Commercial platforms such as those provided by Affymetrix (Barone et al. 2001) and Agilent (Wolber et al. 2006) rely on in situ synthesis of the probes. Affymetrix oligonucleotide arrays consist of 25-mer probes, whereas those of Agilent use longer 60-mer probes. Multiple short oligonucleotides per gene can better discriminate between related sequences, but the longer probes provide better sensitivity. Illumina introduced in 2004 a new microarray technology for quantitative
gene expression profiling on the basis of randomly assembled arrays of beads with each bead carrying a gene-specific probe sequence but multiple copies of each sequence-specific bead in an array (Kuhn et al. 2004). This new platform seems to provide an increased sensitivity compared to the other major commercial microarray platforms, with a larger number of detected genes, and it only needs 25 ng of RNA as starting material for analysis.

Depending on not only the platforms and technologies but also on the RNA source, numerous problems can arise in transcript profiling approaches. One more general challenge is the signal-to-noise ratio, which can be improved with multiple short probes per gene or the same long probe per gene present in multiple copies on the same array. In order to improve interlaboratory comparability, standards for reporting microarray data have been established under MIAME (minimum information about a microarray experiment) (Brazma et al. 2001). This standard contains information required to consistently describe microarray data so that the results derived from its analysis can be independently verified.

Compromised reproducibility of expression profiles is a severe problem that has been meanwhile addressed by a number of studies. However, different conclusions were drawn ranging from good concordance of results across analysis platforms to poor comparability between platforms and laboratories. Recent comparisons of different array platforms (Barnes et al. 2005; Bosotti et al. 2007) revealed that the signal concordance significantly improved with increasing amount of expressed transcript. The concordance was excellent when probes on different platforms could be identified as likely to target the same set of transcripts of a given gene. It appears now that the main factors contributing to result variability are the natural differences between biological samples, rather than the techniques per se. However, variations in sample preparation have been observed when experiments are conducted by different operators. Therefore, the use of automated systems should be envisaged to reduce the “human factor” in technical variability (Raymond et al. 2006).

The challenge of performing microarray studies has today moved from data generation to analysis and interpretation. Analyses restricted to lists of significantly expressed genes with \( p \) values and fold changes are insufficient to fully understand the underlying biology of metabolic adaptations. A single highly regulated gene does not necessarily have an important biological meaning by itself. Therefore, understanding the biological meaning of the many observed gene changes requires their assembly to motifs of regulation. This can be achieved either via cluster analysis as a data-driven approach or by knowledge-based annotation analysis.

Cluster analysis uses statistical algorithms to organize genes according to their similarity in pattern of expression. The result is displayed graphically in an intuitive form for biologists and the highlighted patterns can be interpreted as indications of the cell status. Moreover, coexpression of unknown genes with well-characterized genes can bring suggestions on the functions of genes that are not well described yet.

Annotation-based analysis refers to a set of structured and precisely defined vocabularies, called ontologies, used to characterize genes and gene products. This kind of analysis aims at finding out how genes are involved in different molecular functions, biological processes, and cellular components based on the gene annotation. Thus, ontologies are structured in a form that represents a network of linked terms. Cluster analysis may miss subtle changes, whereas annotation-based analysis appears frequently too restrictive. This is why the combination of both analyses is often used to better interpret a gene expression data set.

A large number of tools are available to the science community for microarray data analysis. Classical software for gene expression experiments usually provides cluster and annotation-based analysis. A new generation of tools also offers metabolic and regulatory pathway analysis based on literature-derived information, enabling to interpret data in a larger context (Joyce and Palmsson 2006; Khatri and Draghici 2005; Weniger et al. 2007; Yi et al. 2006). Despite the fact that the basic standards for reporting microarray analyses are set under MIAME and that a large variety of commercial and public domain software tools for data interpretation are available, there are still limitations in microarray data analysis.

A more specific problem in nutritional applications is the often low signal-to-noise ratio: in contrast to pharmaceutical or toxicological studies in which usually a limited number of target genes show rather robust changes, in nutrition studies transcript levels typically change more subtly, but the number of affected genes is often surprisingly high and this renders interpretation challenging. Therefore, a sound interpretation of the data needs independent confirmation by assessing protein levels by classical techniques or proteomics (see next part of this chapter) in combination with physiological readouts.
MICROARRAY-BASED TRANSCRIPTOMICS IN STUDIES ON HUMAN NUTRITION AND HEALTH

Applications of the different omics technologies appear unlimited when utilizing cells in culture or model organisms, but they are constrained when it comes to studies in humans. Expression profiling at the mRNA level is restricted by the limited availability of vital cells or tissues for analysis. Although tissue samples may be obtained via biopsies, especially in nutrition research, these invasive techniques are restricted in use and require ethical approval in every study—in other words, it is very difficult to obtain a biopsy from a control sample.

Different types of blood cells or even whole blood is therefore generally an interesting source of biological material in human transcriptomic studies. Blood cells respond to dietary intervention, and more interestingly, they have different lifetimes, exhibit different gene expression profiles, and can reach and occupy different body compartments. In particular, peripheral blood mononuclear cells (PBMCs) are sampled for microarray-based identification of candidate mRNA markers in human studies in response to nutritional factors. However, attention should be paid to the result interpretation because PBMCs comprise different kinds of cells (B and T lymphocytes and monocytes), each showing a cell-type-specific gene expression signature. From a technical point of view, care needs to be taken for sample storage and preparation, particularly when using peripheral blood cells for transcriptome analysis. It has been demonstrated that sample handling and prolonged transportation significantly alters gene expression profiles (Debey et al. 2004) and these procedures have to be highly standardized for across-site comparisons. More recently, also whole-blood RNA samples are used for profiling purposes. These require the depletion of globin mRNA in order to detect low-abundance transcripts. Various protocols have recently been written to enhance sensitivity and quality of mRNA detection from whole-blood samples (Field et al. 2007; Ovstebo et al. 2007) but, so far, this has not yet been applied to human nutritional studies.

MICROARRAY-BASED TRANSCRIPTOMICS IN HUMAN NUTRITIONAL INTERVENTION STUDIES

Whole-genome gene expression analysis is increasingly being deployed to assess the efficacy and safety of food ingredients and to evaluate the molecular outcomes of dietary interventions.

Nutrients and genomes interact. Human genetic variation influences nutrient bioavailability and bio-efficacy. An individual’s genome predisposes the organism with regard to the use of nutrients, and—vice versa—the nutrients can significantly alter the expression of the genome. The next-generation transcriptomics technologies, that is, the sequencing-based gene expression analyses are promising means to better determine the molecular mechanisms underlying these interactions and their modification by genetic variation: these techniques enable both the analysis of transcript abundance and its variation among individuals. Nutrigenetics and nutrigenomics are the disciplines addressing these interactions and form the scientific basis for the development of specific diets that could prevent or delay disease and promote health and well-being. This is especially envisaged in chronic diseases because of the lifelong impact of nutrition.

Consequently, a substantial part of these chronic disease-related nutrigenomic/nutrigenetic studies focused on cardiovascular disease, type 2 diabetes, or gastric disorders. While many of the early studies had assumed that single nucleotide polymorphisms (SNPs) were the main source of human genetic variability, an increasing body of evidence suggests the importance of additional layers of variability, including copy number polymorphisms (CNPs) and epigenetic regulation such as DNA methylation. Many complex diseases like irritable bowel disease (Crohn’s disease and ulcerative colitis) have been shown to be related to SNPs on particular chromosomal regions, but are also associated with copy number variation of certain other genes (McCarroll and Altshuler 2007; Shelling and Ferguson 2007). Such discoveries suggest that a detailed description of the genetic background of complex diseases is a challenging but necessary objective in order to better prevent pathological development by, for example, adapted diets.

DNA methylation appears to provide a format for long-term dietary imprinting of the genome (Waterland and Jirtle 2003). Some evidence shows that chronic diseases present in adults are due to persistent adaptations to early-life nutrition. DNA methylation would therefore be directly influenced by dietary methyl supplementation, suggesting that nutritional supplementation may have unexpected adverse consequences on the gene regulation in human, and that well-adapted diets applied already at pre- and postnatal stage may exert a fundamental and long-lasting impact.

Even cognitive development seems to be amenable to genetically counseled nutritional intervention. Studies have shown that nutrients, such as n-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFAs),
can affect brain development and, therefore, the cognitive function. A recent publication showed that the association between breast-feeding and IQ is moderated by a genetic variant in a gene involved in the control of fatty acid pathways (Caspi et al. 2007).

Some gene expression profiling approaches are also used to understand the bioactivity of specific food-derived components and to complement previous epidemiological studies that suggested a potential health benefit. Thus, transcript profiling has been used in nutritional interventions to assess the effect of nutrients. Such studies dealt, for example, with antioxidants with the aim of mimicking the benefits of caloric restriction (Lane et al. 2007); with plant-derived flavonoids like green tea catechins (McLoughlin et al. 2004; Vittal et al. 2004); and with soy isoflavones and flavones (Fuchs et al. 2005a, b; Herzog et al. 2004), which have been shown to provide remarkable biological effects as important as cancer-preventive activity. Other health-beneficial nutrients like polyunsaturated fatty acids (Kitajka et al. 2004; Lapillonne et al. 2004) or micronutrients like zinc (Kindermann et al. 2005; tom Dieck et al. 2005) and vitamin E (Johnson and Manor 2004) have also been studied by transcriptomics to describe their effect on the metabolism. The aim here is to identify an affected set of genes whose regulation illustrates a metabolic adaptation. This type of fundamental discoveries can then be used as a basis for the development of adapted diets focusing on particular (pathological) states of the organism.

Given the degree of complexity of genetic research, it appears evident that a combination of genetics and gene expression experiments applied to the same subjects in the same studies can confer added value, assuming that the analysis tools are ready to integrate the related results. Therefore, the deployment of microarray-based gene expression analyses should and will be more and more complemented by SNP, CNP, or epigenetic studies. Nevertheless, the number of human studies in which only transcript profiling is applied to assess the biological effects of nutritional intervention or to identify markers of health continues to grow.

PROTEOMICS IN NUTRITION AND HEALTH RESEARCH

Proteomics has evolved as an analogue to genomics, from identifying all proteins present in a given sample at a given time to a global molecular analysis platform addressing functional aspects of biological systems (Wilkins et al. 1996). In contrast to the genome, the proteome is highly dynamic and constantly changing in response to the environment of a cell or an organism. Comparing such variations in the proteome enables the discovery of key proteins and the identification of modulated pathways involved, for example, in specific nutrition-related processes. Over the last two decades, proteomics has developed into an established technology for biomarker discovery (Lescuyer et al. 2007; Schrattenholz and Groebe 2007), clinical applications (Mischak et al. 2007), disease profiling and diagnostics (Marko-Varga et al. 2005; Vitzthum et al. 2005), the study of protein interactions (Gingras et al. 2007), and of the dynamics of signaling pathways (Scholten et al. 2006). Nutritional proteomics is an emerging field in which these technologies are applied to nutritional research. It holds great promise to (a) profile and characterize dietary and body proteins, digestion, and absorption, (a) identify biomarkers of nutritional status and health/disease condition, and (c) understand functions of nutrients and other dietary factors in growth, reproduction, and health (Wang et al. 2006a).

In the following, we briefly summarize the main technologies deployed for protein separation, identification, and quantification. Then, we review proteomic studies with a specific focus on nutritional interventions.

PROTEOMICS TECHNOLOGIES

Numerous reviews of proteomic technologies and applications have been published. Most recently, Nature Methods dedicated a special section on mass spectrometry (MS) in proteomics that gives an excellent overview on topics such as large-scale data generation, analysis, and validation (Nesvizhskii et al. 2007), elucidation of cellular networks of protein interactions (Kocher and Superti-Furga 2007), mass spectrometric imaging (Cornett et al. 2007), “top-down” analysis of intact proteins (Siuti and Kelleher 2007), and clinical research perspectives (Beretta 2007).

The proteomics workflow essentially consists of sample preparation and protein/peptide (pre)separation, identification, and quantification. The latter two encompass the complex interface between data generation and processing/validation. Despite tremendous progress at all levels of this workflow, the term “proteome” remains—in contrast to the genome—a theoretical entity, because proteomic studies have to date never revealed an entire proteome. Recent efforts, for example, in Drosophila melanogaster research, catalogued up to 63% of the predicted proteome (Brunner et al. 2007). Coverage in higher organisms, however, rarely reaches
more than 10%, with the numbers for quantified proteins being even smaller (Bantscheff et al. 2007). Nevertheless, open databases (e.g., PRIDE (http://www.ebi.ac.uk/pride) (Jones et al. 2008) or PeptideAtlas (http://www.peptideatlas.org) (Dessiere et al. 2006)) have been established to convert data and results from proteomic experiments into publicly accessible information. The most recent addition of this collective effort to standardize and share protein data is the Human Proteinpedia (http://www.humanproteinpedia.org) (Mathivanan et al. 2008). This portal provides an integrated view of the human proteome, allowing users to contribute and edit proteomic data similar to the online encyclopedia Wikipedia (Giles 2005). Human Proteinpedia can accommodate data from diverse platforms, including yeast two-hybrid screens, MS, peptide/protein arrays, immunohistochemistry, western blots, co-immunoprecipitation, and fluorescence microscopy-type experiments.

For nutritional studies, in vitro samples like cells as well as ex vivo samples such as tissues and body fluids may be suitable. Cultivable primary cells, that is, nontransformed cell lines, should be chosen over cancer cell lines as those have a number of deregulated pathways as compared to normal cells as recently demonstrated by a systems biology-oriented approach integrating transcriptomic and proteomic analysis of buccal epithelial tumor cells (Staab et al. 2007). Cell cultures, however, offer the advantage of virtually unlimited protein supply but the in vitro models may be far from the in vivo situation. Therefore, intestinal epithelial cell lines have been compared at proteomic level to ex vivo recovered gut cells in different cellular stages (Lenaerts et al. 2007).

Ex vivo proteomic samples encompass tissue sections from gut (Lopes et al. 2008; Marvin-Guy et al. 2005), liver (Edvardsson et al. 2003), biliary tract (Kristiansen et al. 2004), and muscle (Gelfi et al. 2006) obtained by resection of biopsies. An important constraint of proteomic sampling for any nutritional study is the demand of being minimally invasive or noninvasive. Therefore, less invasively sampled body fluids like blood plasma (Anderson et al. 2004) and urine (Adachi et al. 2006) are attractive. The plasma proteome is characterized by the highest complexity and the widest dynamic range, but the proteins in blood are by nature relatively soluble. The urinary proteome has revealed an astonishingly high number of intact proteins and is therefore an information-rich proteome source; however, truncation and degradation of urinary proteins add to the complexity.

Extensive sample preseparation, depletion, and enrichment strategies are required due to the complexity of a proteome and the technical limitations of modern MS.

Two-dimensional gel electrophoresis enabled for the first time to separate, visualize, and quantify many proteins simultaneously in one image and paved the way for proteomics (Gorg et al. 2004). The current state-of-the-art methodology is 2D-DIGE, which stands for differential imaging gel electrophoresis (Tonge et al. 2001): the control and case “proteomes” are labeled each with a specific fluorescent dye, then mixed and co-separated, and subsequently analyzed for fluorescent color ratios in a similar way as DNA microarrays. Despite the remarkable improvements of gel-based proteomics thanks to DIGE (Sellers et al. 2007), the gel approach still suffers from (a) difficult automation and thus limited throughput, (b) relatively narrow dynamic range, and (c) a discrimination of proteins with extreme physicochemical properties (size, pI, hydrophobicity).

Therefore, chromatography-based techniques have been developed for protein and peptide (pre)separation. The established term in this context is multidimensional protein identification technology (MudPIT) (Motoyama et al. 2006), also called “shotgun proteomics” approach (reviewed by Wu and MacCoss 2002). Typically, two-dimensional chromatography (ion exchange followed by reversed phase) is online coupled to electrospray ionization (ESI) MS (see below). The approach is based on “proteome” digestion upstream in the workflow and peptide-level separation.

Often, protein and peptide preseparation alone is insufficient for dealing with proteome-scale sample complexity. Therefore, the most abundant proteins may have to be depleted from the sample, that is, specifically be removed by affinity chromatography without affecting the remaining protein composition, especially when analyzing human plasma (Gong et al. 2006) or serum (Bjorhall et al. 2005). The “Equalizer Technology” was described recently (Righetti et al. 2006): a combinatorial library of ligands bound to beads was shown to reduce the concentration differences in human plasma and urine, essentially by binding less of the abundant and more of the rare proteins.

A complementary strategy of gaining access to low-abundant proteins is enrichment of the latter, for example, when targeting subproteomes such as the phospho- or glycoproteome. Various chemical scavengers have been developed to capture phosphopeptides and proteins, such as IMAC (immobilized metal affinity capture), titanium dioxide resins, or alumina.
particles. Recently, Reinders et al. thoroughly compared the performance of these techniques (Reinders and Sickmann 2005). Glycosylated peptides and proteins can be enriched by lectin affinity (Vosseller et al. 2006) or different trapping reactions such as hydrazide chemistry (Sun et al. 2007). Nandi et al. have developed a so-called tagging-via-substrate (TAS) approach for global identification of O-GlcNAc-modified proteins, enabling O-glycosyl enrichment (Nandi et al. 2006).

Key characteristics of a modern mass spectrometer are sensitivity (today femto- to attomolar), mass accuracy (high to low ppm), mass resolution (10,000 to millions), and speed of MS and MS/MS acquisition. MALDI (matrix-assisted laser desorption/ionization) (Tanaka 2003) and ESI (Fenn 2003) are the most popular and powerful methods to produce gas phase ions of proteins and peptides. Different mass analyzers are used in proteomics experiments, such as triple quadrupole (QqQ) instruments, especially for targeted multireaction monitoring (MRM) experiments to, for example, simultaneously quantify dozens of plasma proteins (Anderson and Hunter 2006). Combined with time-of-flight (ToF) or ion-trap (IT) analyzers, they form hybrid systems such as Q–ToF and Q–IT instruments. Fourier transform ion cyclotron resonance mass spectrometers (FT-ICRs) (Nielsen et al. 2005) and the more recently introduced orbitrap system (Makarov et al. 2006) represent the high-end proteomics MS space. FT-ICR instruments offer ultimate resolution (>100,000) and low-ppm mass accuracy that enables “top-down” analysis of intact proteins as opposed to the more frequently employed “bottom-up” approach (Siuti and Kelleher 2007).

Protein quantification can be achieved through staining with protein dyes, and as discussed above, currently the most advanced technology at protein level is 2D-DIGE (Sellers et al. 2007). A further option is to incorporate stable isotopes into proteins and/or peptides in a differential manner (heavy versus light isotope) and to quantify the proteins/peptides by mass spectrometric comparison of the signals derived from the light- and heavy-isotope-labeled sample. These methods have been summarized by the Regnier group (Julka and Regnier 2004) and assessed in real-life scenarios by Wu et al. (2006) and the Heck team (Kolkman et al. 2005). Introduction of the mass labels can also be achieved by metabolic labeling (Beynon and Pratt 2005; de Godoy et al. 2006). This approach is advantageous because of its minimal interference with the biological system. Metabolic labeling is routinely performed with cultured cells ranging from bacteria and yeast to mammalian cells. This has been demonstrated in multicellular organisms such as Caenorhabditis elegans and D. melanogaster (Krijgsveld et al. 2003) and very recently even in rats (McClatchy et al. 2007). Chemical or enzymatic methods must be applied to label ex vivo recovered tissues or fluids. The chemical tagging concept has been introduced by Aebersold et al. under the name ICAT (isotope-labeled affinity tag) (Gygi et al. 1999). The iTRAQ (isotope tags for relative and absolute quantification) method (Ross et al. 2004) offers quadruplex (and soon eight-plex) analysis, that is, four conditions can be compared in one experiment. In view of multiple chemical tagging methods typically performed post-digestion and targeting one amino acid side chain at a time, our group has come up with a new concept termed AniBAL (aniline-benzoic acid labeling): the same tag is introduced into all amino and carboxyl functions already at the protein level (Panchaud et al. 2008). This approach minimizes sample bias, optimizes proteome coverage, and is based on a simple and symmetric chemistry. Bowman et al. have extended the stable-isotope concept to quantitative glycomics by developing a quadruplex derivatization scheme amenable to mass spectral readout (Bowman and Zaia 2007).

Label-free approaches have been developed more recently, based on highly reproducible LC-MS conditions, which allow comparative peptide analysis of complex samples (Old et al. 2005; Ono et al. 2006).

All quantification approaches discussed so far deliver relative quantitative information. Absolute quantification (AQUA) of proteins/peptides was described by Gerber et al. (2003) employing the classical isotope-labeled internal standard approach. The concept of proteotypic peptides takes this strategy to the proteome level by selection of the “best flying” peptide for each protein as a unique identifier (Mallick et al. 2007). Recently, targeted quantification of more than 50 plasma proteins was demonstrated with this approach (Anderson and Hunter 2006). However, design and production of such isotope-labeled reference peptides still needs further improvement for optimal exploitation (Mirzaei et al. 2008).

MS data analysis requires sophisticated software to acquire, store, retrieve, process, validate, and interpret these data and to eventually transform them into useful biological information. While peptide and protein identification and database search programs like Mascot (Perkins et al. 1999), Sequest (Yates et al. 1995), or Phenyx (Colinge et al. 2004) have a long and successful standing (reviewed by Nesvizhskii et al. 2007), entirely new software infrastructures for data processing and validation have been built, such