Reviews

Metabolomics Reviewed: A New "Omics" Platform Technology for Systems Biology and Implications for Natural Products Research

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Metabolomics is the study of global metabolite profiles in a system (cell, tissue, or organism) under a given set of conditions. The analysis of the metabolome is particularly challenging due to the diverse chemical nature of metabolites. Metabolites are the result of the interaction of the system's genome with its environment and are not merely the end product of gene expression but also form part of the regulatory system in an integrated manner. Metabolomics has its roots in early metabolite profiling studies but is now a rapidly expanding area of scientific research in its own right. Metabolomics (or metabonomics) has been labeled one of the new "omics", joining genomics, transcriptomics, and proteomics as a science employed toward the understanding of global systems biology. Metabolomics is fast becoming one of the platform sciences of the "omics", with the majority of the papers in this field having been published only in the last two years. In this review metabolomic methodologies are discussed briefly followed by a more detailed review of the use of metabolomics in integrated applications where metabolomics information has been combined with other "omic" data sets (proteomics, transcriptomics) to enable greater understanding of a biological system. The potential of metabolomics for natural product drug discovery and functional food analysis, primarily as incorporated into broader "omic" data sets, is discussed.

Introduction

Metabolomics is the study of global metabolite profiles in a system (cell, tissue, or organism) under a given set of conditions.[1] Metabolites are the result of the interaction of the system's genome with its environment and are not merely the end product of gene expression but also form part of the regulatory system in an integrated manner. Metabolomics has its roots in early metabolite profiling studies but is now a rapidly expanding area of scientific research in its own right. Metabolomics (or metabonomics) has been labeled one of the new "omics", joining genomics, transcriptomics, and proteomics as a science employed toward the understanding of global systems biology.[2]

The potential of metabolomics is significant, as Mitchell et al. have stated: "Occasionally, a new idea emerges that has the potential to revolutionize an entire field of scientific endeavour. It is now within our grasp to be able to detect subtle perturbations within the phenomenally complex biochemical matrix of living organisms. The discipline of metabonomics promises an all-encompassing approach to understanding total, yet fundamental, changes occurring in disease processes, drug toxicity and cell function."[3] These views have been echoed by others, and this review examines recent studies in metabolomics and highlights integrated approaches, where metabolomics has been studied in combination with proteomics, transcriptomics, or genomics.

There is some overlap in terminology with metabonomics defined as the "quantitative measurement of time-related multiparametaric metabolic responses of multicelluar systems to pathophysiological stimuli or genetic modification",[4] whereas the term metabolomics is broader, though there is significant overlap in methodologies. For simplicity this review uses the term metabolomics.

Metabolite profiling first appeared in the literature in the 1950s and developed throughout the next three decades. Despite this, metabolomics has developed slowly and has only recently become an area of major research interest. The number of publications in the last two years (2003 to the start of September 2005) is more than double the total number of publications on this topic than in the proceeding 23 years combined (Figure 1).

Analytical Techniques and Applications

The first publications generally dealt with the metabolites of specific compounds, such as pharmaceuticals,[5,6] and this type of analysis remains important.[7,8] However these studies soon expanded to include classes of compounds (e.g., catecholamines, oxylipins),[9−11] and more recently full or partial metabolomic analysis has been exploited in a number of disciplines. This work has relied on the improvement of analytical techniques and data handling systems. NMR (nuclear magnetic resonance) spectroscopy and MS (mass spectrometry) techniques are the most frequently used analytical tools for metabolomics, though other analytical techniques have been applied. FTIR (Fourier transform infrared) approaches have been successfully employed to investigate chemical response to plant interspecies competition,[12] to profile salt-stressed tomatoes,[13] and for the analysis of urine for pharmaceutical ADME (absorption, distribution, metabolism, excretion) studies.[14] HPLC-UV (high-performance liquid chromatography) followed by statistical grouping can also be effective and

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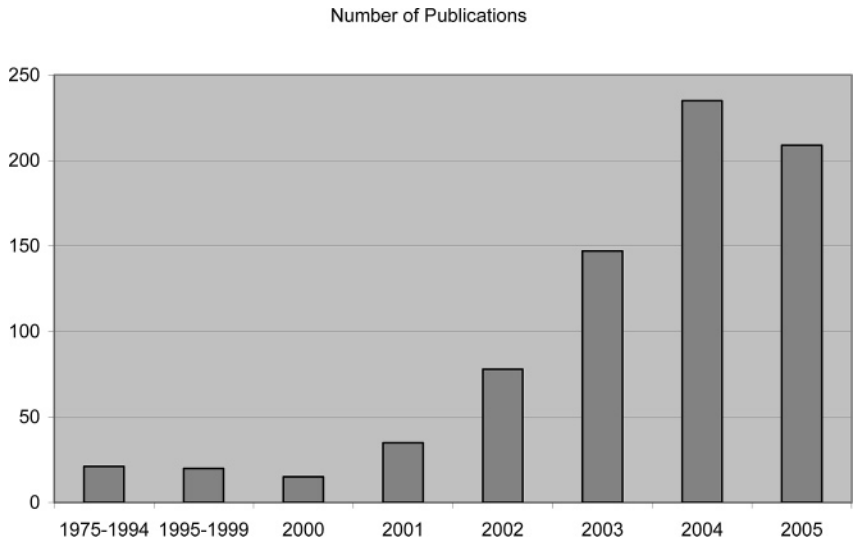

Figure 1. Number of metabolomic related papers published from 1975 to September 2005. Databases searched: Pubmed, Agricola, Analytical Abstracts, CAB Abstracts 1992−2002/07, Current Contents, Food Science and Technology Abstracts (FSTA) 1969−2002/08, ProQuest journal titles, ScienceDirect on 30062005. Search terms used: "metabolomic or metabolomics or metabonomic or metabonomics or metabolite profiling".

has been used to profile phenolic compounds in lignin biosynthesis,[15] to differentiate between patients with hepatitis or liver cancer,[16] and to differentiate transgenic and wild-type plants.[17] Recent advances include localizing metabolite profiles within tissues or cells in real time. This has been achieved for a subset of metabolites within a cell using fluorescence microscopy.[18]

NMR in Metabolomics. NMR is a useful tool in metabolomics, the pros and cons of which have been discussed in some depth.[19−21] Unpurified biofluids are difficult to deal with, and there has been considerable effort to demonstrate techniques to enhance the data quality in both NMR data acquisition and the subsequent statistical analysis.[22−31] One of the most recent has come out of the COMET consortium (The Consortium for Metabonomic Toxicology) and utilizes a non-neural implementation of classical neural net techniques. Time-related and dose-specific effects of toxins on the endogenous urine metabolite pattern were correlated successfully. The authors suggest such models will be useful for building hybrid expert systems to aid in the prediction of toxicology, the ultimate aim of COMET.[28] To enhance interlaboratory reproducibility, orthogonal signal correction has been applied to biofluid analysis and was shown to minimize the influence of inter- and intraspectrometer variation and innate physiological variation.[32] Urine analysis is problematic, and the analysis of plasma is even more challenging. Since the samples are not purified, signals from large proteins may obscure the metabolite profile, but this can be overcome by utilizing the difference in molecular behavior between large and small molecules. A NMR-based study on toxin-induced changes in lipoprotein profiles utilzed spin diffusion differences to remove the effects of the small molecule.[33] In the analogous experiment, signals from large molecules (proteins in plasma) were reduced by spin−echo techniques to enhance metabolite analysis.[34] NMR analysis has been shown to be sufficiently robust to be of use in clinical studies,[35] and variations in age, gender, and diet have been investigated to further examine the capability of the technique to detect variation due to additional factors.[36,37] These results suggest that these NMR-based techniques can be applied to systems that differ due to genetics and environment.

While 1D ^{1}H NMR is the most used method in NMR metabolomics, there have been reports of 2D techniques being used. Urine profiles from cattle treated with anabolic steroids have been analyzed by HMBC techniques with linear discriminant analysis.[38] 2D *J*-resolved spectroscopy has been used to generate decoupled 1D spectra for the analysis of embryogenesis.[23] Despite its relative insensitivity, ^{13}C NMR has also been applied to metabolomics in the study of brain tissue.[39] It is also possible to examine the metabolome of intact tissue or cells through the use of magic angle spinning techniques.[22,40,41]

Mass Spectrometry. GC (gas chromatography) and LC-MS (liquid chromatography−mass spectrometry) are also important analytical techniques for metabolomic analysis. Several MS techniques have been used in metabolomic approaches. GC-MS techniques have been applied in several studies.[42−44] Biomarkers of adrenarche were examined in a large study of 400 subjects using GC-MS,[45] and 2D GC-TOFMS (time-of-flight mass spectrometry) has been used in the analysis of metabolites in ryegrass.[46] It has been noted that LC-MS profiling followed by statistical grouping is an effective approach to metabolomics and has been identified in a number of studies including analysis of rat urine in drug development.[47] LC-MS is increasingly used and is the subject of a recent review that considers the use of HPLC-MS in metabolomics and the potential of future developments.[48] Direct infusion techniques have also been effectively utilzed for metabolome analysis.[49] For example, direct infusion ESI (electrospray ionization) TOFMS has been used for the analysis of complex mixtures and has been used successfully in plant analysis.[50,51] Similarly, the utility of capillary liquid chromatography ESITOF has been demonstrated in the analysis of *Arabidopsis* secondary metabolites.[52] ESIMS ion trap instruments have been used to detect oligosaccharides, glycosides, amino sugars, amino acids, and sugar nucleotides in plant tissue[53] and for profiling rat urine in a study of heavy metal toxicity.[54] CE (capillary electrophoresis) ESIMS has been used to identify sugar nucleotides, and applications of this technique for bacterial metabolomics have been discussed.[55] The potential of FTICRMS (Fourier transform ion cyclotron resonance mass spectrometry) has been discussed in a recent review.[56]

Peak identification tools such as the NIST database are useful for specific compound identification in GC-MS, but multivariate data analysis can be equally well applied to these data sets as to that of NMR. Plant scientists have

developed the program MSFACTS, which can import and reformat large data sets. The program was demonstrated using GC-MS data generated from different tissues in a legume.[57] Developments in this area continue. For example, a new visualization tool named COMPSARI has been released recently, which is designed to facilitate the identification of small differences in MS data.[58] The authors demonstrated the validity of their technique analyzing wild-type and knockout yeast strains, with the software correctly grouping extracts and identifying the molecules involved. COMPSARI has been made freely available.

Combining Techniques. NMR and HPLC-MS techniques are complementary. However, very few studies have used more than one analytical technique. LC-MS and NMR have been used in combination to detect disease biomarkers in urine, which led to an 84% success rate in distinguishing patients with interstitial cystitis compared to bacterial cystitis.[59] The advantage of NMR is that it is nondestructive, relatively quick, and cheap (after the initial costs of installation). The disadvantage is its relative insensitivity compared to MS. LC-MS technology is also improving, with good sensitivity, but LC procedures often require relatively long run times (20 min to 2 h). New advances in HPLC including coupled columns and columns using sub-2 μm packing combined with high operating pressures may change this. In a recent example, run times were reduced from 50 to 5 min, making high-throughput metabolomic analysis by LC a real possibility.[48] Similarly, the development of instrumentation such as cylindrical ion trap array MS, which simultaneously can analyze four samples, but potentially many more, may speed up LC-MS analysis times.[60]

Applications

Metabolomics has been an application-driven science. A natural extension of early single metabolite analysis was the application of metabolomics to identify disease biomarkers.[16,40,61−66] Specific examples to indicate the range of approaches include the successful diagnosis of coronary heart disease,[67] the identification of Lesch-Nyhan syndrome (a serious mental disorder),[42] the correct discrimination of women with epithelial ovarian cancer compared to control subjects,[68] and the investigation of host−pathogen interactions in plants.[69] Analysis of blood spots has enabled the detection of inborn errors of metabolism,[70] and the techniques have also been applied to shellfish to identify novel biomarkers for withering syndrome (a disease of abalone).[71]

Some of the groundbreaking work has been done in the field of human health. Early studies used both NMR and MS analysis to profile biofluids such as rat urine.[36,72,73] Profiling of biofluids has become a major focus of research in several areas, with one of the ultimate goals being the promise of individualized drug therapy,[74] and, as such, profiling samples for indications of drug toxicity is one area that is particularly well developed.[14,75−78] Possibly the largest concerted effort in this area is the COMET project. The project is constructing databases and metabolic models of drug toxicity using ca. 100 000 ^{1}H NMR spectra of biofluids from animals treated with model toxins.[79] This consortia combines Nicholson's group at Imperial College in London and six pharmaceutical companies, who have so far invested tens of millions of dollars toward the development of metabolomic-based toxicity screens for drug development.[2]

The plant research community has also been extremely active in the development of metabolomics. Plants accumulate a very large number of metabolites with important functions in plant ecology and in the protection against stress conditions. Metabolomics offers additional methods to analyze these complex interactions.[80] There has been concentrated interest in metabolomics as applied to functional genomics and systems biology, and this has been the subject of a number of reviews.[81−83] The plant research community has been sufficiently active in the area to warrant a special edition in *Phytochemistry* devoted to metabolomics (Volume 62, Issue 6, March 2003).

Metabolomic analysis is sensitive enough to pick up subtle differences between a wild-type organism and its transgenic counterpart. For example, HPLC analysis has been successfully used to differentiate wild-type and transgenic alfalfa plants of the same genetic background in stem phenolic profiles.[15] MS and NMR metabolomics techniques have also been applied to differentiate wild-type versus transgenic organisms in tobacco,[84] potatoes,[85] and maize.[86]

GC-MS metabolomics approaches have been used to discriminate between two related genotypes of *Arabidopsis* (the strains only differed in their chloroplast and mitochondria but were otherwise genetically identical); the study correctly classified the two genotypes and also their progeny.[44] MOA (mode of action) studies for herbicides have also demonstrated the utility of NMR spectroscopy followed by neural network analysis to identify herbicides with potentially novel MOAs.[87]

Profiling for strain differences (chemotaxonomy) has been employed in plant systems where, for example, NMR analysis was used to discriminate between *Ephedra* species.[88] Chemotaxonomic uses are not restricted to plants, and these techniques have been used to identify three varieties of shellfish.[71] Demonstrating that this technique is useful in microbial studies as well, Bundy et al. were able to successfully discriminate between nonpathogenic strains of *Bacillus cereus* and pathogenic strains (isolated from meningitis patients).[89] Similarly, it has been demonstrated that direct infusion MS is a useful technique in distinguishing between fungal strains.[90]

Metabolomics in Systems Biology. Integration of genomics, metabolomics, transcriptomics, and proteomics is a goal of systems biology and has been much discussed.[91−98] An emerging area of interest is that of nutrigenomics (the study of the effect of nutrition and environmental factors on an individual's genome). In nutrigenomics there was early recognition of the potential to integrate the "omic" sciences.[99−107] Metabolomics is recognized as an important aspect of this integration.[108] There have been several reviews published in this area, including assessments of the potential of plant "omics" technologies to impact the food industry;[82] food safety issues as it relates to GM food;[109] and, in particular, assessing unexpected outcomes of GM (genetically modified) technology.[110−112] Diet and nutrigenomics have also been considered in direct relation to diseases such as cancer.[113−117] Nutrigenomics is seen as an important tool for maintenance and improvement of human health and recently received a boost in Europe with the formation of "The European Nutrigenomics Organisation", which has been awarded a 17.3 million euro investment for study over a six-year period.[118]

The effect of diet on the human or animal metabolome has been the subject of much discussion.[103,119,120] There have been some studies, including an analysis of the effect of human dietary intervention with soy isoflavones by NMR.[121] The metabolomic effect of epicatechin (a suspected bioactive component of tea) was analyzed in rats.[122] A study

published in 2005 highlighted the potential of metabolomics in nutrition research. A clear difference between the metabolome patterns (NMR-based study) of chamomile tea drinkers before and after dosing was demonstrated, and the results suggested that the effects of tea drinking lasted for at least two weeks post-dosing.[123] Similar to the concept of individualized medicine, nutrigenomics suggests the possibility of individualized diets. Such integration would optimize the health of individuals by giving them informed choices about the foods that are optimum to maintain health and open opportunities for the development of specific functional foods to facilitate this.

It has also been postulated that population and toxicogenomic studies should benefit from the combination of "omics", by allowing connections to be drawn between environmental factors in terms of diet and exposure to toxicity and environmental induced diseases.[124,125]

Data Set Integration and Correlation

The potential of integrating the "omics" has prompted an increase in reports correlating two or more data sets.[126−135] Linking the "omics" is problematic for several reasons, including translational control of mRNA (i.e., not all expressed mRNA is converted to protein)[136] and processes on the transcription and translational level that lead to the formation of isoenzymes, which, when combined with poor enzyme specificity, can result in multiple metabolites from one gene set.[137] Another complication is the differential timing of these events.[98] Background differences (due to environment and genetics) between individuals in a study have also been of concern. For example, growth conditions were shown to have considerable impact on gene expression and either obscure or enhance differences between samples (tomato wild-type and hexokinase transformants).[138] However, these difficulties can be overcome. Metabolomics and microarrays have been used to explore phenotypic characteristics that are controlled by both genomic and environmental constraints.[139] In recent work by Kant et al., proteinase inhibitor activity, gene transcription, and metabolomic analysis were integrated in the study of host−pathogen interactions (tomato−spider mite).[69] This integrated approach was also used by Kleno et al. to identify potential biomarkers for hepatotoxicity in rats. Correlations from liver mRNA, liver proteome, and metabolome analysis of serum were shown to correspond to changes in glucose, lipid metabolism, and oxidative stress responses.[140] Proteomics and metabolomics have been combined in a study of the cardiovascular system where the authors noted that: "Importantly, the simultaneous assessment of protein and metabolite changes translated purely descriptive proteomic and metabolomic profiles into a functional context and provided important insights into pathophysiological mechanisms that would not have been obtained by other techniques."[141] Similarly, recent proteomics, transcriptomics, and metabolomics analysis of human brain tissue investigating schizophrenia identified that almost half of the altered proteins were associated with mitochondrial function and oxidative stress response, which was mirrored by transcriptome and metabolome perturbations. Cluster analysis identified 90% of schizophrenia patients from controls, providing important new information about mitochondrial dysfunction in schizophrenia.[142] New techniques are being developed to facilitate this integration of the "omics", for example, the sequential extraction of metabolites, proteins, and RNA from the same *Arabidopsis* leaf sample.[143]

Integration has many potential benefits. A recent report details how transcriptome analysis in combination with metabolite analysis resulted in an improved strain of the pharmaceutically important lovastatin-producing fungus (*Aspergillus terreus*).[144] While this study was limited to metabolite analysis of a few compounds, it demonstrates the potential to identify genes whose functions are not obvious. Such profiling also has potential in medical or dietary studies to identify genes that are linked to particular health outcomes. Rats given a high-fat diet were shown to overexpress certain genes that led to insulin insensitivity, and metabolic profiling of 36 acylcarnitine species revealed a unique decrease in one lipid-derived metabolite. This metabolite could be linked to the expression of a particular gene in the liver.[145] A wider study was undertaken in 2003 on dairy cows where the effect of diet on both milk lipid content and lipogenic mRNA abundance was correlated to examine the links between diet and gene expression.[146]

Again the plant research community has been at the forefront of this research. In a recent experiment designed to facilitate the identification of 20 metabolically important gene clusters in plant cells deliberate perturbation of gene expression by jasmonate showed good correlation between mRNA and metabolome expression.[147] In a separate study, concurrent analysis of gene expression and metabolomics revealed few differences between red and green forms of *Perilla frutescens*. The differences lay in the levels of anthocyanins, which could be correlated to differences in several genes, which were then potentially linked to the regulatory network of anthocyanin expression.[148] In an investigation of different potato tubers and pairwise transcript−metabolite correlation, 571 out of 26 616 possible pairs showed strong correlations.[149] This sort of integration has been extended to the detection of silent mutations in plants.[150] Silent plant phenotypes (where there is no obvious biochemical marker for knockouts) have been detected by subtle differences in metabolite patterns.[151] Similarly, comparing the metabolome of wild-type and enzyme-inactivated organisms can identify endogenous substrates.[152]

Use of the technique has also been demonstrated in nonmammalian animal systems. Heat shock protein expression has also been shown to correlate well to heat stress metabolome signatures in fish,[153] demonstrating the potential application of metabolomics to economically important industries such as aquaculture.

Models and Data Analysis

Data analysis and sensibly applied statistical tools are of crucial importance if the field of metabolomics is to live up to its potential. A number of books and articles have been written discussing statistics use and data analysis in metabolomics and the importance of good experimental design.[30,154−159] Different approaches have been undertaken. In one study the use of NMR protocols and multivariate statistical batch processing was examined for consistency over six different centers and was shown to be sufficiently robust to generate comparable results across each center.[77] Raamsdonk et al. have developed a methodology termed FANCY (functional analysis by co-responses in yeast) for studying silent mutations in yeast that allows the statistical correlation between data sets to reveal the role of silent genes.[150]

Data analysis and the lack of generally available databases are areas of concern in metabolomics. As yet, there are no comparable systems to those available for the study of genomics and proteomics. However, progress is being made and there have been several approaches to draw data

together in a meaningful way. A number of tools have been developed for plant scientists. Lange et al. recently reviewed these tools, which included a number of online databases and introduced their own tool, BioPathAt, for the model plant *Arabidopsis thaliana*.[160] ArMet is a database constructed to allow handling of "omics" data for plant metabolomics,[161] while Thimm and colleagues are developing a modular tool called MAPMAN to integrate "omics" data and demonstrated its use in a study of *Arabidopsis*.[162] Also under development for the plant community is IRIS (International Rice Information System), which was originally employed to handle germplasm genealogy but is being extended to include genetic mapping, genome annotation, genotype, mutant, transcriptome, proteome, and metabolomic data.[163] There are also bacterial databases, including one formed around *E. coli* K-12.[164] In terms of human studies, one of the largest medical applications is the COMET project, which, as mentioned previously, is constructing databases and metabolic models of drug toxicity.[79] Xirasager et al. have recently described their Chemical Effects in Biological Systems (CEBS) knowledge base that is being designed for systems biology data and leverages other open source efforts including Micro-Array Gene Expression Object Model and the Proteomics Experiment Data Repository.[165] HumanCyc is a genome-based view of human nutrition that associates essential dietary requirements with a set of metabolic pathways. It may facilitate analysis of gene expression, proteomics, and metabolomic data sets and utilzes an online tool called Omics Viewer, which can be searched based on metabolites.[166] Ultimately the aim of such integrated models is to predict metabolic flux under specific conditions.[167]

The integration of "omics" data is challenging and may require new models to adequately address these challenges. One such example is the "omic space" framework proposed last year.[168] Toyoda et al. describe "omics space" as a dimensionless framework where interactions are defined on the basis of their positions within the coordinate system. They describe a system named the "Genome to Phenome Superhighway (GPS)", which integrates databases and visualizes "omic" interactions to allow a "totalomic" approach to systems biology.

Metabolomics for Natural Products Research

Application of these new models and the integration of "omics" data have potential in drug discovery and MOA studies. Natural product drug discovery and functional food analysis will also benefit from these techniques. One of the often-perceived hurdles involved with natural product drug discovery is the possibility of redundancy, that is, working on multiple samples with the same active agent. There are numerous "dereplication" procedures that have been developed to address this problem, particularly by researchers focusing on microbial extracts. Many of these involve HPLC and/or MS/NMR analysis. The use of a metabolomic approach, with appropriate statistical analysis, has the potential to allow rapid analysis of these complex data. A recent example is the analysis of partially purified plant and sponge extracts by NMR and the subsequent clustering by different statistical methods.[169] The authors used tree clustering, K-means clustering, and multidimensional scaling to analyze data without prior knowledge. They concluded that statistically valid results could be obtained using tree clustering and K-means clustering, in combination, allowing them to cluster extracts with similar components at different concentration levels. NMR clustering techniques have also been used to cluster the spectra of crude plant extracts to determine the biochemical MOA for herbicides.[170] In this study, 27 herbicidal compounds with 19 different MOAs were applied to plants for 24 h before an aqueous extract of the plant was taken. Artificial neural network analysis was employed to analyze the ^{1}H NMR spectroscopic data of these extracts. Treated samples were easily distinguished from untreated samples, and, with the use of an appropriate training set, many different MOAs were distinguished for the commercially available compounds tested. The same approach would be of use in cellular-based assays used in pharmaceutics and natural products research to discover MOA. This approach has been recently demonstrated for an herbal medicine. A medicinal herb extract (*Anoectochilus formosanus*: a popular folk medicine with anticancer activity) was compared to a single compound drug in MCF-7 cells by metabolomic and transcriptomic analysis, and a similar level of gene expression regulation was observed in both, suggesting a similar MOA.[171] Determination of MOAs continues to be problematic in many circumstances, and such integrated approaches as this may, if not determine the target, reduce the number of possibilities to be considered. Thus an anticancer agent detected from whole cell screening may show metabolomics effects closer to those produced by known tubulin inhibitors than known kinase inhibitors, reducing the number of therapeutic molecular targets that must be investigated.

Challenges for Metabolomics

There are a number of challenges for the developing science of metabolomics. For the science to develop in the same way as genomics and proteomics, standard ontology must be adopted. Metabolomics ontology and experimental reporting standards have been proposed by the Metabolomics Society, among others. Details of these requirements were put forward in a recent article in *Nature Biotechnology*.[172] Adoption of standards will make it easier to integrate the large amount of data that will be generated in metabolomic experiments and will enhance reproducibility and credibility of metabolomics data.

Much of the initial work on metabolomics has been of a qualitative nature, often the result of statistical model analysis rather than assessment of individual metabolite flux. This level of qualification and quantification is of increasing importance, as the significance of more biosynthetic pathways is elucidated. GC-MS applications have benefited from large databases such as the NIST database. For LC-MS these databases are still in their infancy, and the commonly used ESI techniques are much more dependent on multiple variables than the equivalent EI techniques employed for GC-MS. For both, MS applications standards will be needed to quantitate specific metabolites. For human or animal studies this may be achieved by the synthesis of specific isotopically labeled metabolites. In plant metabolomic experiments this will be more problematic, since the metabolite number and the structural diversity of the metabolites that plants produce is much greater. There are a number of "lessons learnt" from proteomics that can be applied to metabolomics including reproducibility, issues which are being considered.[32,77] The adoption of standards and reporting of all experimental conditions will assist in this area.

Identification of specific metabolites in complex mixtures by NMR is also problematic. However, it has been demonstrated that many common metabolites can be identified by the application of 2D NMR experiments. A recent

example is the identification of more than 50 metabolites in a lettuce leaf extract.[173] The next challenge for metabolomics by NMR is quantitation, and there have been some studies in this area, including examples demonstrating the accuracy for algal toxins.[174] While proton NMR can be quantitative, the resolution is not as good in the less quantitative carbon dimension, creating problems for separating compounds. More research on quantitation by 2D NMR needs to be done, and it remains to be seen if 2D NMR will offer the level of quantitation required for rapid metabolomic analysis.

Data analysis and integration remains problematic. As has been discussed, there have been considerable advances in this field, but more work is needed to define the limits of current systems and to offer the possibility of better systems. As the scientific knowledge of metabolic pathways increases (including the gene, the proteins, and metabolites involved), this can be expected to improve.

Conclusion

There are challenges in terms of technology, experimental design, data analysis, and data integration that will impact on the field of metabolomics and its application to systems biology. Given the potential of this science and interest in this field, as demonstrated by the increase in the number of publications, there is evidently belief in the scientific community that metabolomics has real potential to facilitate the study of systems biology.

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