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Chemistry meets nutrition: Toward a systems biological description of human metabolism*

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Abstract: Chemistry was already pioneered by ancient Egyptians up to 4000 years ago. Despite its age, chemistry is by no means a dying scientific discipline. The different branches of study within chemistry have infiltrated all fields of life science research, and nobody can work in this area without using chemistry in the broadest sense. The present article is a personal view on how chemistry supports life science research, in particular in the field of nutrition and metabolism research. It provides insight into how chemistry, in close collaboration with life science research, helps to fill the gaps between our current fragmentary understanding and the comprehensive knowledge required for better understanding the molecular details of metabolism, health and disease, and aging. The most important contributions of the chemical disciplines to these studies with respect to a systems biological description of human nutrition and metabolism will be outlined.

Keywords: coherent anti-Stokes Raman scattering (CARS); metabolism; metabolomics; metabonomics; nutriomics; nutrition; fluxomics; Raman microscopy; reactomics.

INTRODUCTION

Living organisms "require a continuous influx of free energy to maintain order in a universe bent on maximizing disorder. Metabolism is the overall process through which living systems acquire and utilize the free energy they need to carry out their various functions. They do so by coupling the exergonic reactions of nutrient oxidation to the endergonic processes required to maintain the living state such as the performance of mechanical work, the active transport of molecules against concentration gradients, and the biosynthesis of complex molecules" [1].

This fundamental view on metabolism, cited from the renowned textbook *Biochemistry* by Donald and Judith G. Voet, holds true not only at first glance. But there is more to the metabolism of highly organized organisms, such as vertebrates or plants, than a simple coupling of exergonic reactions and endergonic processes for acquiring and harnessing free energy. The fates of nutrients are manifold: they range from complete oxidation to breakdown in a series of enzymatic reactions to intermediates that are used as precursors in the synthesis of other biological molecules. As a consequence, every second several hundreds of different enzymatic reactions take place and hundreds of metabolites are formed in each cell of the organism.

Due to its complexity, metabolism needs tight organization and strict temporal coordination. Metabolic reactions are therefore organized into a series of consecutive enzymatic reactions that pro-

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duce specific products. These individual "metabolic pathways" comprise a complex "metabolic network" (see figure in Supplementary Information). To ensure on demand formation and delivery of specific metabolic intermediates and products, the flux of energy and the formation of products within the metabolic network is well coordinated by an even more complex "regulatory network". This network consists of several distinct "signaling pathways" which interact with each other and regulate themselves via feedback loops or feedforward control.

THE METABOLOME AND ITS ORGANIZATION

The complete set of small-molecule (<1500 Da) metabolites (metabolic intermediates, signaling molecules, secondary metabolites, etc.) to be found within in a cell, organ, or organism is referred to as its metabolome [2]. Like the transcriptome and the proteome, the metabolome is dynamically changing from second to second. According to a first draft published in 2007 [2], the human metabolome consists of about 7100 small molecules that have been catalogued and characterized as more than 2100 metabolites, 1500 drugs, and 3500 food components. At the time of writing, the Human Metabolome Database (version 2.5) contained already more than 7900 metabolite entries, including both water- and lipid-soluble metabolites as well as metabolites that would be regarded as either abundant (>1 μM) or relatively rare (<1 nM) [3,4].

Besides the numerous metabolites of the metabolic network comprised by the metabolome, the situation in higher-organized organisms, such as plants and animals, is made even more complex by the fact that metabolic networks, as well as regulatory networks, act at and across different hierarchical levels of organization. By this way, an intricate multilayer system of chemical communication is comprised, involving, inter alia, metabolites as chemical information units.

The first level of organization is the cell itself. Each individual cell is characterized by a specific metabolite fingerprint that may change to another characteristic profile in response to exogenous stimuli or uptake of nutrients. This occurs, for example, when macrophages, the phagocytic cells of the vertebrate immune systems [5], respond to bacterial lipopolysaccharide. This stimulus activates distinct signaling pathways via binding to a cell surface receptor called Toll-like receptor [6]. Activation of the receptor then results in an inflammatory response but also in changes in the metabolic flux [7]. Macrophages activated in that manner are essentially glycolytic cells compared to nonactivated cells. Although this condition appears to be simple at first sight, the metabolic flux distributions at the cellular level are complex to such an extent that so far no description of cellular metabolism has even come close to a comprehensive representation of the complete metabolome or metabolic network of a vertebrate cell.

In addition to the cellular level, metabolic and regulatory networks exist between the different cells within tissues. Hence, the situation is complicated, because metabolic and regulatory networks act across the different types of specialized cells. Thus, each cell type of a distinct tissue has its own metabolic and regulatory system, changing in time and depending on the various tasks of the cells within the tissue. A recent study by Kosteli and colleagues, for example, provided insight into the complexity of such regulatory and metabolic systems, which exhibit both changes in metabolism as well as cellular composition [8,9]. The authors analyzed macrophage dynamics and gene expression over a period of gradual weight loss in obese mice on a calorie-restricted diet. Initially, macrophage numbers in adipose tissues increased and decreased after two months. The initial increase in macrophage accumulation was not accompanied by a concomitant increase in inflammatory response. The initial diet-induced increase in adipose tissue macrophages was associated with higher concentrations of free fatty acids in the serum, which were released from adipocytes by lipolysis. Interestingly, adipose tissue lipolysis positively correlated with macrophage numbers. Hence, macrophages respond to acute changes in lipolysis and migrate to adipose tissues, where they phagocytose excess lipids without causing inflammation. As triglyceride stores are depleted and lipolysis decreases during ongoing weight loss, macrophages leave the adipose tissue and its metabolic function is maintained.

The separation of distinct metabolic pathways into specialized tissues (i.e., organs) in plants and vertebrates allows for better adoption of distinct metabolic pathways to the needs of the organism in response to its environment. Well-known examples are the restriction of gluconeogenesis or ketone body formation to the liver and subsequent transport of glucose or ketone bodies to the tissues, which use these molecules for energy supply. This restriction of distinct metabolic pathways to certain types of tissues requires another level of organization of metabolism. This third level of organization is the systemic level in which metabolic networks exist between different types of organs. Well-known, "classic" examples of simple cross-organ metabolic networks are the Cori cycle* and the glucose-alanine cycle**. Again, the different metabolic and regulatory networks at this level interact with each other and also with those of other levels of organization, finally comprising the complex systemic metabolic system.

NUTRITION AND THE METABOLOME

Unlike the human genome, which is almost invariant and fully sequenced, the human metabolome is changing from second to second. More than 7900 endogenous metabolites have been identified so far in the human body [3], but many more metabolites may exist. Unraveling the metabolome is demanding because metabolites are not to be found consistently in any given tissue or biofluid. Differences in metabolite profiles are due to severely different functions of tissues and biofluids, but also to the several hundreds of compounds living systems ingest. Finally, the complexity of the metabolome depends on what the organism ingests but also on its genetic background because mutations and polymorphisms may influence enzyme activity and thus modulate the metabolic flux in the body. It is therefore likely that each individual has its own metabolic fingerprint not only characterized by a distinct set of metabolites but also by the different amounts of individual metabolites and the relative composition of the metabolome. Thus, the concentration of each metabolite contributes to the uniqueness of a metabolome and almost no metabolome of a human will be identical to another.

But what is the incentive to bring to light (i) which compounds are in our diet, (ii) which metabolites are formed from these compounds, and (iii) in which quantities these metabolites appear in the different cells and tissues of our body? The answer is simply that nutrients are not just a source of energy. Many small molecules taken up with our daily food or as drugs, as well as the metabolites and xenometabolites that are formed from these compounds within the body, interfere with the regulatory network or interact directly with metabolic enzymes, thus modulating their activity and metabolite formation. Such regulatory naturally occurring molecules are often referred to as "bioactive compounds". In principle, such compounds act in a similar way as synthetic or natural drugs do. For example, they act as ligands of nuclear receptors or interfere with enzymes by modulating their catalytic activity. By this bioactive food components are embedded into both the metabolic as well as the regulatory network. Discordance between bioactive food components and the metabolic and regulatory network may result in chronic pathologic conditions, whereas such compounds, on the other hand, may as well prevent age- and nutrition-related diseases.

Despite many years of research, surprisingly little is known about where, when, and by which enzymes these metabolites are formed and how they interact with cellular and systemic metabolic or signaling processes. It is therefore not clear for most small molecules entering our body where and how they are metabolized and what impact they may have on the regulatory and the metabolic network and finally on health and vitality.

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*Metabolic pathway in which lactate produced by anaerobic glycolysis in muscles moves to the liver and is converted to glucose, which then returns to muscles and is converted back to lactate.

**Metabolic pathway which enables pyruvate and glutamate to be removed from muscles and find their way to the liver. Glucose is regenerated from pyruvate and then returned to muscles: the energetic burden of gluconeogenesis is thus imposed on the liver instead of muscles.

Diet-induced changes in metabolic flux and nutrition-related diseases

The body requires carbohydrates, lipids, proteins, vitamins, and minerals to maintain health, and to produce hormones and chemicals that are necessary for the proper function of organs. Tight and simultaneous coordination of the metabolic and regulatory network utilizing all of these compounds is a prerequisite to maintain vitality and health of a living organism. Permanent or recurrent distortions in the normal metabolic flux and the regulatory circuit may result in pathologic metabolic conditions, such as atherosclerosis, obesity, diabetes, etc., similar to inherited metabolic disorders, in which distinct metabolic pathways are disrupted due to mutations in genes encoding enzymes of the corresponding pathway (Fig. 1). Typical chronic metabolic disorder, which may be caused by a misbalance and a consecutive change in normal uptake of nutrients, and an accompanying disturbance of the metabolic flux in the human body are diabetes and atherosclerosis [10], which are caused by, for example, continuous

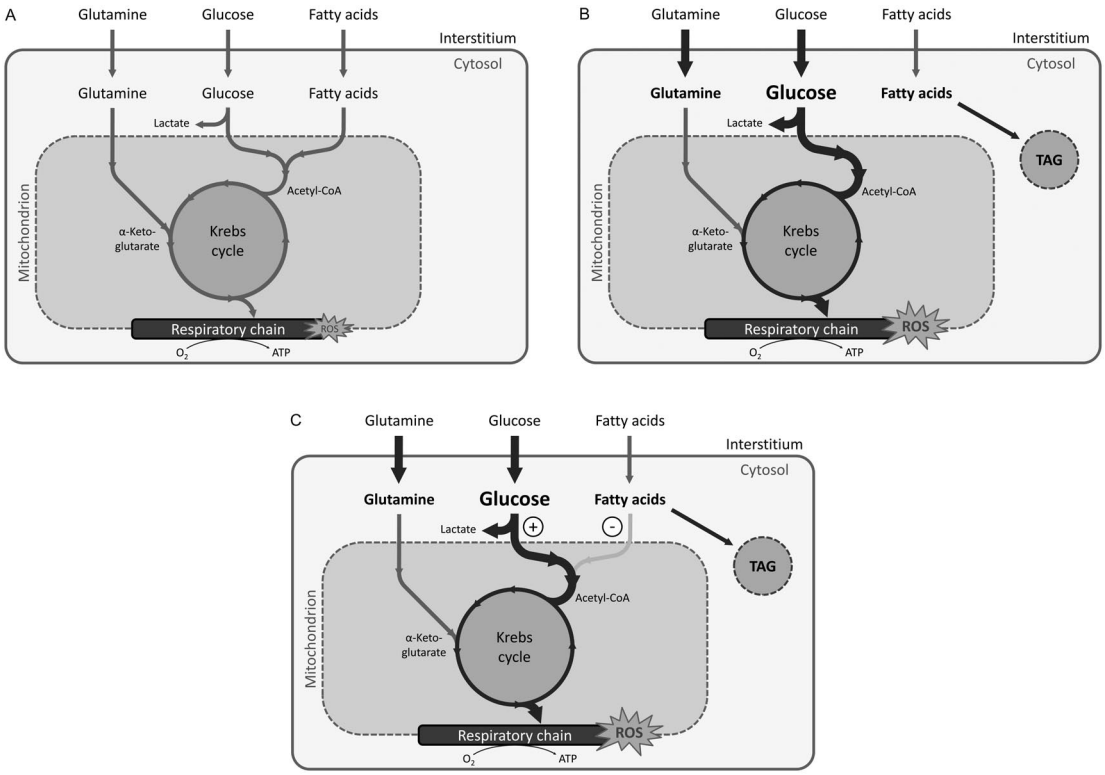

Fig. 1 Changes in metabolic flux in macrophages due to defects of central metabolic pathways or in response to environmental stimuli. (A) In resting macrophages, mitochondria oxidize glucose, glutamine, and fatty acids and produce ATP via the electron flux in the respiratory chain. The continuous leakage of electrons from the respiratory chain results in weak production of mitochondrial ROS. (B) Mutations causing defects in the metabolic oxidation or the import of acyl-CoA into mitochondria would result in a switch to increased utilization of glucose, increased ROS production and simultaneously shuttle fatty acids as triglycerides into lipid storage. (C) Following activation with lipopolysaccharide (symbolized by (+)), oxidation of glucose is strongly increased contrarily to oxidation of glutamine and fatty acids. Consequently, the flow of electrons in the respiratory chain is affected, leading to an increase of mitochondrial production of ROS in addition to ATP. Similar increases in glucose oxidation occur if uptake or oxidation of fatty acids in mitochondria is decreased by specific inhibitors, for example, etomoxir (symbolized by (–)), which efficiently inhibits import of acyl-CoA into mitochondria by irreversibly blocking O-carnitine palmitoyltransferase-1 (CPT1) [51]. The figures were adapted from [52].

high ingestion of carbohydrates and lipids resulting in a disturbed glucose and lipid metabolism. Interestingly, although many factors are known to contribute to these diseases, the molecular mechanisms and metabolic processes leading to the disease are only incompletely understood.

Disease prevention through diet and nutrients

Obesity and heart attacks are major health problems in the United States and other Western industrialized countries. Therefore, most dietary recommendations are aimed at preventing these two diseases. Obesity is caused by eating more calories than the body actually burns. Obesity, in turn, can cause many secondary diseases such as heart disease, diabetes, liver disease, arthritis, high blood pressure, gout, gallstones, and certain cancers [10]. To lose weight or maintain a healthy weight is a good way to prevent or at least reduce the risk of developing these metabolic disorders [11]. For the reduction of these risk factors, weight loss exceeding 10–20 % of the initial body weight is probably necessary, which can be achieved through either conventional lifestyle measures or more drastic interventions such as bariatric surgery. However, the increasing average body mass index (BMI) in Western industrialized countries and the growing incidence in developing diseases of civilization, such as diabetes and atherosclerosis, show that this is not a trivial task. Indeed, many, if not most, obese people fail to reduce and to maintain lower body weight in the long term.

It has been shown that certain well-balanced diets, such as the Mediterranean diet, constitute a means of improving the risk factors for cardiovascular disease [11], or, in the case of the Asian diet, correlate with a lower incidence of cardiovascular disease. Both diets are rich in vegetables, and usually consumption of meat is low. It has therefore been suggested that these diets contain particular ingredients with health-improving benefit similar to that of ω-3 fatty acids which are held responsible for the reduced risk of developing cardiovascular events in Inuits despite their diet rich in fats and their high BMI [12]. Indeed, many compounds in our diet exhibiting health-improving properties in cell or animal models have been identified and isolated. Together with the increasing evidence of the diseases of civilization, this raised the demand for developing health beneficial food enriched in ingredients, such as flavonoids, plant sterols, phytoestrogens, monoterpenes, and others, as well as dietary supplements, such as vitamin E.

In many cases, the health beneficial claims have arisen from epidemiologic observations that populations, in which diets rich in particular ingredients are consumed, have reduced risks of developing certain metabolic diseases. Renowned examples are consumption of soy rich in phytoestrogens in Asia and marine fish rich in ω-3 fatty acids by Inuits [12,13]. However, scientific confirmation of health benefits is required to make the development of food enriched in potentially health beneficial ingredients reasonable and justifiable. Further, careful scientific evaluation is necessary before long-term consumption of such enriched food or of food supplements can sensibly be recommended because all natural-occurring compounds bear the risk of harmful side effects. To evaluate beneficial properties as well as harmful side effects of such food ingredients, pure compounds are required for dietary studies in appropriate animal models as well as for functional studies in cell culture systems.

In this respect, chemistry has made and will continue to make important contributions: Chemical analyses are required for quantitating the content of the compounds of interest in normal and in enriched food as well as to investigate bioavailability and metabolism in the human body. Further, efficient and cost-effective isolation procedures or synthesis strategies allow for producing enriched food and supplements that may help prevent disease and aging, on a large scale economically.

CHEMISTRY MEETS NUTRITIONAL SYSTEMS BIOLOGY

Systems biology in terms of nutrition and metabolism aims at unraveling the complexity and interactivity of the metabolic and regulatory circuits described above at all organizational levels. However, despite impressive technological advances in recent years, we still lack the tools and standards that

allow reproducible analysis, absolute quantification, and subcellular localization of all the individual small-molecular components of the complex system comprising a living system at reasonable costs, sufficient sensitivity, and throughput.

In the following, I will provide a short perspective on how chemistry helps researchers in the field of nutrition and metabolism on their way toward a systems biological description of human metabolism and dietary impact on it. However, this perspective is more or less a personal view and makes no claim to be complete. It is restricted to (i) quantitative high-throughput and high-resolution analysis and quantification of metabolites and metabolic fluxes, (ii) natural compounds and drug discovery, and (iii) development of technologies and tools for enhanced microscopy. Another limitation of the present outline, which is due to the lack of expertise of the author in this respect, is the absence of a projection on how chemistry may help. The author, however, is confident that creative chemists will find solutions and will, in close collaboration with life science researchers, fill in the gaps between our current understanding and the knowledge required for understanding the molecular details of metabolism, health and disease, and aging.

Unraveling metabolomes and understanding that metabolism requires different "omics" approaches

The "omics" has in many respects revolutionized life science research in general and nutrition research in particular. "Omics" techniques on the whole are likely to assist the quest for a better understanding of how nutrition causes diseases and how nutritional approaches can be used to prevent aging and disease. One of the challenges of systems biology and functional genomics is to integrate proteomic, transcriptomic, and metabolomic information to yield a more comprehensive picture of living organisms. Undoubtedly, chemistry has contributed to all "omics" technologies, for example, by providing strategies for on-chip synthesis of nucleic acid probes for DNA microarray production as well as by preparation procedures and analytical tools, such as mass spectrometry, for proteomics. However, without the approaches described in the following sections, systems biology would lack one of its most vital building blocks. Therefore, I have focused on metabolomics, metabonomics, fluxomics, and reactomics that are required for a better in-depth understanding of the metabolic network as a whole.

Metabolomics and metabonomics

Comprehensive knowledge about the metabolome and its change in time in response to various stimuli is a prerequisite for systems biology. The analytical tools used for studying the metabolome, which have emerged in recent years, will be explained in order to highlight the value chemistry has added and continues to add to this field of nutrition research.

Metabolomics is the "systematic study of the unique chemical fingerprints that specific cellular processes leave behind"—specifically, the study of their small-molecule metabolite profiles [14]. The metabolome represents the collectivity of metabolites in a biological cell, tissue, organ, or organism, which are the end products of cellular processes [3]. Thus, while mRNA gene expression data and proteomic analyses do not tell the whole story of what might be happening in a cell, metabolic profiling can give an instantaneous snapshot of the physiology of that cell, tissue, or organism, which is consequently its phenotype. Metabonomics is defined as "the quantitative measurement of the dynamic multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modification" [14]. There has been some disagreement over the exact use of "metabolomics" and "metabonomics". It is generally agreed that the difference between the two terms is not related to the choice of different analytical platforms, although metabonomics is usually more associated with NMR spectroscopy and metabolomics with mass spectrometry-based techniques. While there is still no absolute agreement, there is growing consensus that metabolomics places a greater emphasis on metabolic profiling at a cellular or organ level and is primarily concerned with normal endogenous metabolism. Metabonomics, on the other hand, extends metabolic profiling to include information about per-

turbations of metabolism caused by environmental factors (including diet, toxins, and drugs), disease processes, and the involvement of extragenomic influences, such as gut microflora.

In practice, within the field of human disease research there is still no definite distinction in the way both terms are used, and they are often used synonymously. In the following, the term "metabolomics" is synonymously used with "metabonomics", although the author refers to metabolism as a system that is influenced by exogenous factors, such as diet in general and specific food ingredients, such as secondary plant metabolites, in particular.

The increasing number of publications regarding metabolome studies reflects the importance of metabolomics; recent milestones are the draft description of the human metabolome [2], LIPID MAPS (see section "Unraveling metabolomes requires interdisciplinary, coordinated multi-centered efforts and high levels of standardization") [15], and the genome-wide association of 163 metabolic traits measured in human blood from more than 1800 individuals [16]. However, much progress has also been achieved in other research areas, such as investigating metabolomes of plants and yeast [17,18], response to drugs [19], and virus infection [20], to name only a few.

However, many obstacles to metabolite identification and quantification still persist [21–23]. For example, the complexity and inhomogeneity of crude cell and tissue lysates impede detection of reaction products, surmounting these often demands additional chromatographic steps that lower sensitivity and throughput of the analysis. Although these methods are effective, they obstruct the procedures, make automation difficult, and introduce additional experimental variables. Reproducible methods for sample preparation are a major concern, as intracellular metabolites can be quickly degraded or otherwise chemically modified. Another obstacle is that metabolomic techniques involving mass spectrometry are able to detect thousands of chemical compounds in a single experiment, but it is usually a time-consuming and demanding process to positively identify each of the compounds. Further, the sensitivity and spatial resolution of the currently available methods need to be improved to track the behavior of metabolites with the proteins that use these compounds as substrates, products, or ligands. These drawbacks have often led to the wide variation in the reproducibility of metabolomic measurements. Enhanced sensitivity and resolution would also allow for tighter links between observations of structure and function. Finally, no solutions exist on how to illuminate complex interaction systems, such as the interaction of the gut microbiome with the cells of the gut, which involves dynamic and complex exchange and mutual reactions of small molecules.

Fluxomics

Systems biology is based on the notion that the whole is greater than the sum of its parts. The ultimate goal of systems biology is to predict the complexity of the whole system as well as its behavior on the basis of the components involved and the stimuli in effect. Among the main "omics" technologies, metabolomics plays an important role in bridging the phenotype–genotype gap, since it amplifies changes in the proteome and provides a more detailed representation of the phenotype of an organism than other methods. Unfortunately, knowledge of the complete set of metabolites, the metabolome, is not enough to predict the phenotype, because it represents a steady state only. This holds especially true for cells of complex organisms in which the distinct metabolic processes are finely regulated and interconnected, in particular in response to the many exogenous stimuli. In these cells, quantitative knowledge of intracellular fluxes is required for a comprehensive characterization of metabolic networks and their functional operation. These intracellular fluxes cannot be detected directly, but can be estimated by interpretation of stable isotope patterns in metabolites. Moreover, analysis of these fluxes by means of metabolic control theories offers a potentially unifying, holistic paradigm to explain the regulation of cellular metabolism and ultimately systemic metabolism. Fluxomics can also play a more and more important role in drug discovery.

Fluxomics is the field of research that deals with these dynamic changes of small molecules within a cell over time, i.e., large-scale metabolic flux analysis [24]. Consequently, it is an "omics" term that essentially is a neologism of flux balance analysis (FBA) with a wider and more systematic scope.

Different stable isotope labeling strategies are required for fluxome characterization. Fluxomics experiments are conducted by, for example, culturing cells in medium with carbon substrates containing mixtures of different carbon isotopes, if required distinct isotopes at different positions in the molecule are used. Labeled carbon atoms are processed and incorporated into different metabolites that can be isolated and analyzed by using mass spectrometry to determine the chemical pathways that were used to synthesize the final molecule. By this approach, intracellular functional data can be obtained in terms of the current turnover of metabolites in specific metabolic pathways.

Although metabolic flux measurements have been successfully used in many different biological systems and fluxomics has become a reproducible method, three major concerns limit the application of this technique. A major obstacle of fluxomics is that the level of detail that can be obtained is very limited. At each moment hundreds to thousands of reactions take place in any living system of interest. But methods for metabolic flux analysis are only capable of distinguishing flux splits for 20–30 points in a metabolic network. This means that comprehensive metabolic flux analyses at the systemic level are almost not possible. Another problem is that at least some knowledge of the metabolic network being studied is required before flux analyses can be conducted. An in silico challenge is to construct the most comprehensive model to represent the metabolism of a specific cell. Usually, stoichiometric models are used to estimate intracellular fluxes from experimental data, a process strongly susceptible to modeling biases.

Reactomics

Reactomics is the "omics" study of the reactome that is essentially the totality of biological reactions of an organism [25]. The need for methods that grant access to a reactome was demonstrated by the recent deciphering of an alternative glycolytic pathway in rapidly proliferating cells, such as cancer cells [26]. Proliferating cells require altered metabolism to efficiently utilize nutrients such as glucose. The M2 isoform of pyruvate kinase promotes the metabolism of glucose by aerobic glycolysis. Paradoxically, decreased pyruvate kinase enzyme activity accompanies the expression of this enzyme in rapidly dividing cancer cells. Vander Heiden and colleagues demonstrated that phosphorenolpyruvate, the substrate for pyruvate kinase, can act as a phosphate donor as it participates in the phosphorylation of the glycolytic enzyme phosphoglycerate mutase. This reaction yields pyruvate in the absence of pyruvate kinase activity and provides an alternate glycolytic pathway that uncouples ATP production from phosphoenolpyruvate-mediated phosphotransfer.

Whereas metabolomics provides new insights into the metabolic steady state of a living system under a given set of environmental parameters, or in response to a parameter change, reactomics allows for unraveling the enzymes and accompanied metabolic pathways which are active in a cell of interest. However, functionally associating the metabolic profile obtained with the enzymes and pathways responsible still relies heavily on sequencing-based metabolome reconstructions. There is thus a great need for methods to causally link metabolites to cognate enzymes, which, in addition to delivering systemic descriptions of metabolic responses to exogenous stimuli, simultaneously provide annotation of the enzymes involved.

Such a powerful new method of assessing the functionality of the active proteins in any given cell was described by a group around Manuel Ferrer in October 2009 as the "reactome array" [27], which forges the link between genome and metabolome and establishes a global metabolic phenotype of an extract of cells, tissues, or multicellular organisms all at once. The reactome array of nearly 2500 metabolites and other substrate compounds tethered to a glass slide would allow scientists to assess the functionality of hundreds of active proteins simultaneously and would constitute a generic tool for metabolic phenotyping of cells and annotation of proteins. For the array, the authors synthesized 1676 metabolites known from the Kyoto Encyclopedia of Genes and Genomes (KEGG) database [28] to collectively represent central metabolic pathways and 807 other substrate molecules [27]. The generic structure of these reactome metabolites involved three linked components: the enzyme substrate-metabolite, the quenched dye, and the linker used to immobilize the complex to a glass slide. The dye,

which is initially inactive, becomes activated when an enzyme acts on a substrate; this reaction generates a fluorescent signal and supplies a quantitative measure of enzyme activity. Additionally, because the substrate linkers physically trap reactive enzymes, proteins can be isolated and identified using mass spectrometry.

When scientists first read about the new technique, they claimed that "it seemed too good to be true", and indeed, over the last several months, biochemists have questioned the validity of this new technique. *Science* therefore decided to post an "Editorial Expression of Concern" that appeared in the journal on 8 January 2010 [29]. The questions focus in particular on the synthesis of the dye-labeled metabolites that are central to the technique. In addition, the spectroscopic data the authors cite in support of their claim were not posted at the time of publication, despite the authors' indication in the supplemental material. In response to inquiries from *Science*, the authors have provided new descriptions of the synthetic methods that differ substantially from those in their original article. Nevertheless, the study has provided a first idea how the gap between quickly advancing genome data and functional annotation that is lagging further and further behind can be closed, and may build the basis for an accurate and comprehensive way to study cellular metabolism. In addition, such a tool could also lead to the discovery of interesting reactions for biotechnological applications. Hence, chemists are asked to bring this "ultimate" tool for globally analyzing enzymatic reactions in cells and tissues up to the task in hand.

Unraveling metabolomes requires interdisciplinary, coordinated multi-centered efforts and high levels of standardization

The multi-institutional LIPID Metabolites and Pathways Strategy (LIPID MAPS) consortium created in 2003 and led by Edward A. Dennis (University of California, San Diego) is a good example for demonstrating the technical demand of deciphering metabolomes [15]. The LIPID MAPS consortium was initially funded by a large-scale collaborative research grant ("glue grant") from the NIH National Institute of General Medical Sciences of about USD 35 million over a five-year period. The aim of LIPID MAPS for this grant period was to develop the requisite technology and conduct an integrated research program that will establish lipidomics, a subset of metabolomics, as a fully developed research field [30]. The long-term objective of LIPID MAPS is to identify and quantitate all of the major—and many minor—lipid species in mammalian cells, as well as to record the changes in these species in response to perturbation [15]. The ultimate goal is to improve the understanding of lipid metabolism and the active role lipids play in diseases such as diabetes, atherosclerosis, cancer, and other lipid-associated diseases in order to facilitate development of more effective treatments.

To achieve this, LIPID MAPS has brought together a group of 12 scientists who operate 10 cores and 5 bridges in close collaboration over 6 focus areas: (i) administration, (ii) informatics, (iii) cell biology, (iv) lipid detection and quantification, (v) lipid synthesis and characterization, and (vi) lipidomics. Further, a common set of biochemical and cell culture protocols was defined which insure that reproducible experiments are carried out in each of the consortium laboratories and that the data collected by one unit are directly comparable to data collected by another. Six of these cores developed the techniques to precisely determine the quantity of a given class of lipids. To increase the reproducibility, to insure that data from different lipid classes could be compared, and to cross-check data, each of the core labs was equipped with an identical liquid chromatography/mass spectroscopy system. The data thus generated have been collected through a laboratory information management system, stored in databases, and analyzed by Informatics experts.

The three primary goals of the consortium were (i) to separate and detect all of the lipids in a specific cell and discover and characterize any novel lipids that may be present, (ii) to quantitate each of the lipid metabolites present and quantitate the changes in their levels and location during cellular function, and (iii) to define the biochemical pathways for each lipid and develop lipid maps, which define the interaction networks.

Since its inception, Lipid MAPS has made great strides toward defining the lipidome, an inventory of the thousands of individual lipid molecular species, in mouse macrophages. However, LIPID MAPS nicely illustrates how ambitious, technically as well as financially, it is to achieve the aim of unraveling a subset of the metabolome of a given cell.

Annotating genes requires natural compounds and drug discovery

Chemical analytics clearly contribute to our understanding of human metabolism. But, is defining the human metabolomes in this restricted way sufficient for our understanding how nutrition contributes to health and aging? There are estimates that the human metabolome is in the magnitude of thousands of metabolites, but there are likely more than 20 000 for microbes and 200 000 for plants [31]. The human gut, for example, is home to over a kilogram of microbes. At present, the metabolites arising from this human microbiome have not been catalogued and are not part of the current definition of the human metabolome. At present, researchers expect that the microbiome is not only responsible for a coordinated digestion but also contributes, for example, actively to the immune system of the human body, thus causing lasting changes in the human metabolism [22].

In addition to the compounds produced in the microbiome of our gut, the human body is increasingly confronted with ingredients contained in exotic vegetables and fruits, which, owing to increasing globalization, become available worldwide as part of our nutrition. Such compounds may be beneficial for maintaining human health and slowing aging processes, but they may also bear the risk of having even harmful effects. In order to study how the compounds consumed with exotic fruits and vegetables as well as the ones produced by the gut's microbes are metabolized in our body and to understand their regulatory effects, we need careful analytics to, in a first step, identify these ingredients and, in a second step, to isolate or synthesize them in sufficient amount and at reasonable cost for functional studies in vitro and in animal models.

This is another important contribution that can be made by chemistry: (i) to identify and quantitate these natural compounds and (ii) to isolate sufficient amounts of pure compounds for functional studies in vitro and in vivo or alternatively synthesize sufficient amounts. Further, the identification of natural compounds produced by plants and microbes may help identify new inhibitors, antagonists and activators of enzymes, nuclear factors, etc. that help researchers to better understand metabolism and regulatory processes in our body. They may also become an inspiration and valuable source for the discovery of drugs for preventing disease and slowing aging processes.

Understanding metabolism requires cellular localization of metabolites and enzymatic reactions

Besides comprehensive knowledge about the small-molecule composition, the metabolome, of a cell, it is of particular interest to localize these small molecules within the cell as well as the respective enzymes with corresponding reactions; in the case of such dynamic processes at the cellular level, real-time measurements are desirable. In order to facilitate investigation of individual metabolite species, label-free techniques are of particular interest. In the following, selected exemplary insights into some recent developments are given that allow for investigating cellular localization and bear the potential for analyzing cellular reactions and product formation at cellular resolution.

Raman microscopic imaging

With recent technological advances, Raman microscopic imaging was successfully applied to cell biological research and is now accepted as a new type of microscopy, complementary to more routinely used fluorescence-based approaches [32]. The intrinsic spectral information of a molecule conveys information on its molecular composition on the sub-micrometer level and can be used to reconstruct images based on spectral parameters. Here it is possible to identify, for example, early-stage cancer

[33,34], prokaryotic [35–37], and eukaryotic microorganisms [38,39]. Multivariate chemometric algorithms are applied to extract the relevant spectral information used for classification [40,41].

In terms of cellular imaging, Raman microscopy has been used to monitor changes associated with stress introduced by cytotoxins [42], ingestion of amino acids [43], or to track uptake and metabolism dynamics of drug delivery systems [44–46]. In order to distinguish a certain molecule species of interest from the cellular environment and increase sensitivity and specificity, stable isotopic labels, such as deuterium, can be introduced [45,47]. The possibility to image uptake dynamics as well as intracellular fate of individual compounds noninvasively offers great potential.

Along with the increase of diet-related diseases, it is important to gain insight into the metabolism of biological molecules in the body and also at the cellular level. In this context, lipids, such as fatty acids, triglycerides, or cholesterol, are of particular interest since their deposition, for example, in the walls of arteries contributes to cardiovascular diseases, the most common cause of death worldwide [48,49]. In arteries, lipids are taken up by macrophages, the phagocytic cells of the immune system [5]. Macrophages engulf and digest cellular debris and pathogens. Within arterial walls, macrophages play an important role in regulating the metabolism of lipids that are taken up by receptor-mediated endocytosis and non-endocytotic pathways [48,49]. Once ingested, lipids are either metabolized, stored in lipid droplets [50–53], or exported via transporters and acceptor-proteins for transport to the liver in a process termed "reverse cholesterol transport" [54]. If lipid uptake exceeds the potential of both latter processes to deal with the accumulating lipids, the continuous uptake of lipids becomes cytotoxic and may lead to cell death [48,49]. The subsequent deposition of cell-derived lipids and macrophage-derived debris in arterial walls contributes to the development of vulnerable or unstable plaques, rupture of which can cause sudden fatal incidents, such as a heart attack or stroke [48,49].

Recently, first results on the subcellular distribution of individual fatty acids using label-free Raman microscopy have been presented [55]. Figure 2 shows Raman microscopic images of a macrophage incubated with 400 μM serum albumin-bound oleic acid for 3 h. The Raman image was generated using an unmixing algorithm as previously described [55]. The general protein distribution is plotted in blue, indicating size and shape of the cell body. The green regions are very likely associated with organelle rich structures, e.g., the endoplasmic reticulum (ER) or Golgi apparatus, which are usu-

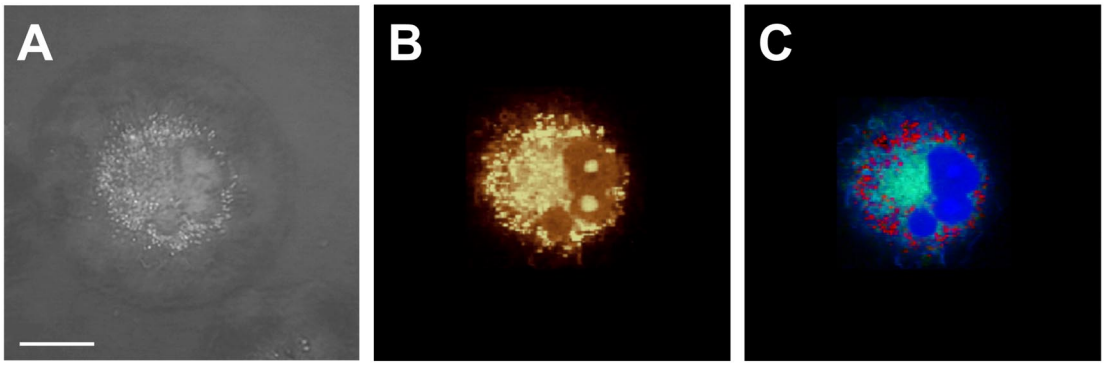

Fig. 2 Raman microscopic analysis of a fatty-acid-derived macrophage foam cell. (A) Bright field image of a macrophage incubated with serum albumin-conjugated oleic acid-d_{34} for 3 h at 400 μM. (B) Corresponding Raman image depicted from the scattering intensities of the CH stretching vibrations between 2800 to 3100 cm^{-1}. (C) Raman image reconstructed by a spectral decomposition algorithm from the scattering intensities of the CD stretching vibrations between 2100 to 2300 cm^{-1} showing the intracellular distribution of the oleic acid-d_{34} in red. The general protein distribution is plotted in blue, reflecting size and shape of the cell body. Turquoise regions are likely associated with organelle-rich structures, for example, endoplasmic reticulum (ER) or Golgi apparatus. The bar indicates 20 μM.

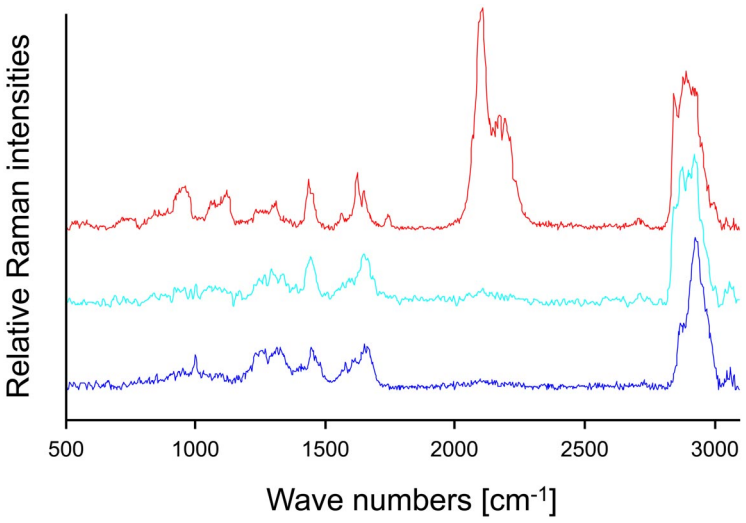

Fig. 3 Raman spectra of a fatty-acid-derived macrophage foam cell. Raman spectra correspond to the Raman microscopic images shown in Fig. 3. The same color scheme as in Fig. 3 is used.

ally located around the nucleus. Areas with high lipid content, here the regions of interest, are depicted in red. Raman spectra corresponding to the pictures shown in Fig. 2 are depicted in Fig. 3.

The work by Matthäus and colleagues demonstrates the feasibility of Raman microscopic imaging for monitoring uptake of lipid species such as different types of fatty acids in cultured cells. Furthermore, the use of isotopic labels allows for visualization of cellular incorporation and subsequent processing specifically for a given type of lipids over time. By avoiding the introduction of fluorescence tags, the chemical properties of the molecules of interest remain unaltered, and consequently the uptake dynamics, kinetics, and efficiencies of different types of fatty acids, such as palmitic, oleic, and arachidonic acid, can be studied directly and without any bias. By referring the Raman intensities to the overall intensities of the Raman signals originating from the cell's proteins or other lipids, it is also possible to draw quantitative conclusions [55,56].

Raman microscopic images are of excellent quality; however, recording these images is time-consuming. At a spatial resolution of 500 nm, a single picture requires a Raman map consisting of more than 10 000 spectra with exposure times of 250 ms per pixel. This results in about 40 min of total acquisition time. Utilizing the CARS (coherent anti-Stokes Raman scattering) effect can overcome these time limitations [55], and may allow for live-cell imaging [56]. First results have been obtained using non-labeled as well as stable isotope labeled compounds [56]. The total acquisition time does not exceed 10 s for all frames.

In addition to superior spectroscopic equipment providing, for example, higher resolution, the lack of appropriate deuterium compounds is currently limiting the approach. Using appropriate synthesis strategies, such compounds can be produced in sufficient amount and at reasonable cost for cellular localization studies. Another important enhancement is to combine this technique with fluorescent labels to allow, for example, colocalization studies of metabolites and cellular protein structures.

Fluorescence-based microscopic imaging

Cellular localization of small molecules can be either obtained by using dyes that specifically bind to other compounds in the cell, such as BODIPY or nile red for detecting accumulated neutral lipids, or that are covalently linked to the molecule of interest. Both approaches bear the risk that the fluorophore influences the biological behavior of the molecule of interest, because it may modify its biophysical

characteristics or because the fluorescence label is similar to or even larger in size than the molecule of interest

Nevertheless, it is of continuing interest to have fluorophores at hand that allow exact localization of metabolites in cells. An interesting approach is to develop sensitive fluorophores that allow visualization of the formation of particular metabolites in living or fixed cells. In this respect, fluorescent sensors are of particular interest that detect the formation of reactive oxygen species (ROS) or nitric oxide, molecules which act as efficient signaling molecules and exhibit different functions depending on the cellular compartment in which they are formed. Another promising task is to visualize the interactions of different small molecules with each other or of small molecules with proteins, such as enzymes, nuclear factors, or by transporter, utilizing techniques such as fluorescence resonance energy transfer (FRET).

Only a brief introduction to this topic is provided here because a more detailed description is beyond the scope of this article.

FINAL REMARKS

Systems biology is the systematic and coordinated study of complex biological systems (i) by investigating the individual components of cellular networks as well as their interactions, (ii) by applying high-throughput and whole-system ("omics") methods, and (iii) by integrating computational algorithms with experimental efforts. As outlined here, chemistry plays an important part in deciphering biological systems using systems biological approaches. However, the manifold contributions of the chemical sciences to nutrition and metabolism research cannot be exhausted in a single essay. Obviously, in addition to the topics outlined here, chemistry supports life science researchers of the "omics" era in many other ways: (i) enhancing the chemistry and technology behind unraveling DNA and RNA sequences using next-generation sequencing, (ii) characterizing protein sequences and identifying protein modifications using spectrometric and spectroscopic approaches, and (iii) preparing probes for DNA microarrays or nanoparticles using innovative organic synthesis approaches are only a few to name. The aim of the present outline was to demonstrate that life science research requires support by creative and innovative chemists because the chemical sciences bear the potential to solve many of the problems life science researchers are facing when they work on unraveling the hidden secrets of metabolism and metabolic disorders. The close interdisciplinary collaboration between life scientists and chemists from all disciplines will definitively help to advance our understanding of cellular and systemic metabolic as well as regulatory processes in the human body. Thus, our progress toward the ultimate goal of preventing disease, improving life quality, and extending life expectancy by paving the way to a systems biological description of nutrition and nutrition-related metabolism is promoted. From this cross-section, a novel hybrid discipline arises which may be termed "nutriomics" what is essentially a new "omics" study where researchers adopt genomics, transcriptomics, proteomics, and interactomics technologies in combination with metabolomics, fluxomics, and reactomics approaches to investigate how the human body deals with the many compounds in our diet.

SUPPLEMENTARY INFORMATION

Supplementary information is available online (doi:10.1351/PAC-CON-10-11-06).

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