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Metabolomics and systems biology: making sense of the soup

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Novel techniques for acquiring metabolomics data continue to emerge. Such data require proper storage in suitably configured databases, which then permit one to establish the size of microbial metabolomes (hundreds of major metabolites) and allow the nature, organisation and control of metabolic networks to be investigated. A variety of algorithms for metabolic network reconstruction coupled to suitable modelling algorithms are the ground substances for the development of metabolic network and systems biology. Even qualitative models of metabolic networks, when subject to stoichiometric constraints, can prove highly informative, and are the first step to the quantitative models, which alone can allow the true representation of complex biochemical systems.

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Abbreviations

FTIR	Fourier transform infrared (spectrometry)
GC-MS	gas chromatography-mass spectrometry
GC-TOF	gas chromatography time-of-flight (mass spectrometry)
LC-MS	liquid chromatography-mass spectrometry
MCA	metabolic control analysis

Introduction

'There is an epoch in the growth of a science during which facts accumulate faster than theories can accommodate them.'

Medawar, P. (1982) in *Pluto's Republic*. Oxford University Press, Oxford, p. 29.

'But one thing is clear: to understand the whole one must study the whole.'

Kacser, H. (1986) in The Organization of cell metabolism, ed., G.R. Welch and J.S. Clegg, Plenum Press, New York, p. 327.

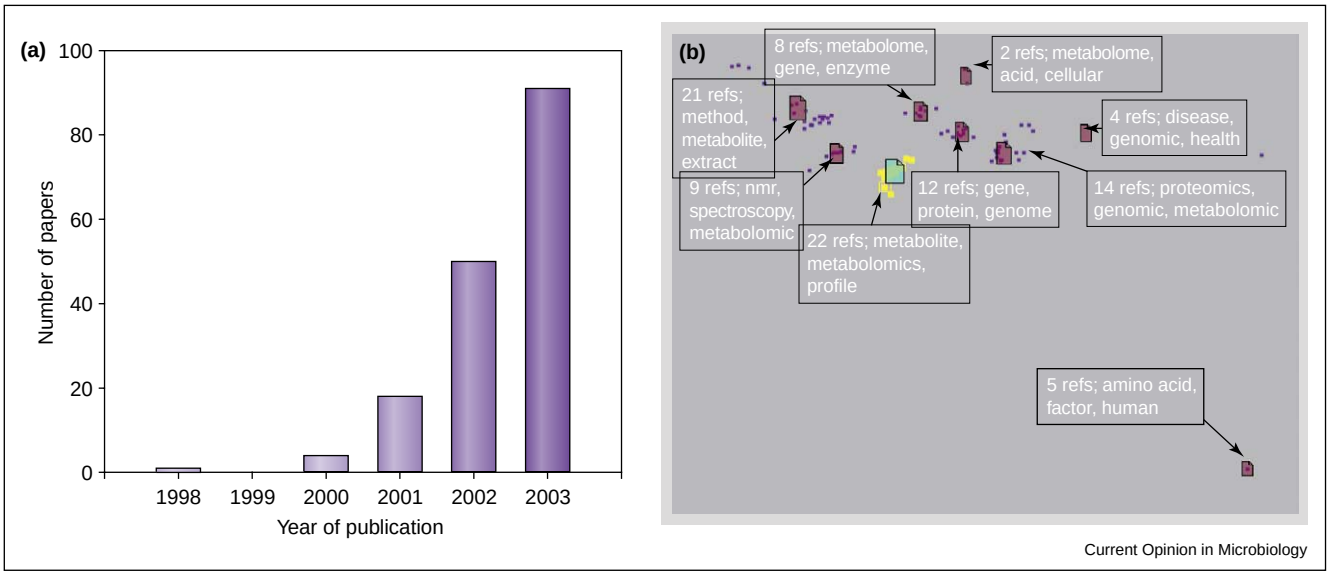
It is becoming increasingly apparent that our ability to generate large quantities of metabolomic or metabolic profiling data will help to open up many previously inaccessible areas of biology. However, such data are merely the inputs or ground substance to systems designed to provide understanding or knowledge, and affecting this may require substantial changes in the conventional and purely hypothesis-dependent, reductionist thinking that has heretofore been common [1••,2]. Metabolomics is a burgeoning field (Figure 1), which produces voluminous data that, like other 'omics' data, should be seen as a resource that contributes specifically to the former half of an iterative cycle of hypothesis-generating and hypothesis-testing phases [2,3•,4,5••] (Figure 2).

In this review, I highlight advances in the way we both gather and use metabolomic data for the large-scale reconstruction of biological systems and for the generation of both testable hypotheses and the predictive models that lie at the heart of systems biology. To 'make sense of the soup', we should concentrate on the questions 'who is there', 'who are they talking to', 'how are metabolic networks organized' and 'what does it mean for our understanding of the cell or organism?' For readers seeking recent reviews of the general field of metabolomics, the following useful surveys have appeared during the review period [6•–8•,9••,10•,11,12•–14•,15••,16•].

Getting the data

The first requirement is to have available techniques that are as comprehensive as possible for metabolic analyses. As the chemistry of different metabolites is very heterogeneous, isolating and measuring them all together ('true metabolomics') is very hard, and most metabolic studies are really 'metabolic profiling' of subsets of chemical classes [8•]. Favoured instrumental methods in different fields (especially plants and microbes versus animals) have differed, largely for historical reasons, but there is increasing convergence to use as many as possible for all samples as their complementarity is appreciated. As well as increasingly refined gas chromatography-mass spectrometry (GC-MS) methods, especially those using gas chromatography time-of-flight mass spectrometry (GC-TOF) instruments that allow much better deconvolution than do most GC-MS instruments because they can record spectra, and thus sample, very quickly, several recent methods appear to show promise. Fourier-transform ion cyclotron resonance (FT-ICR) mass spectrometry [17,18•] is a very high-resolution mass spectral method (10^5 – 10^6, with mass accuracy better than 1 ppm), which allows separation and empirical formula measurement of potentially thousands of metabolites

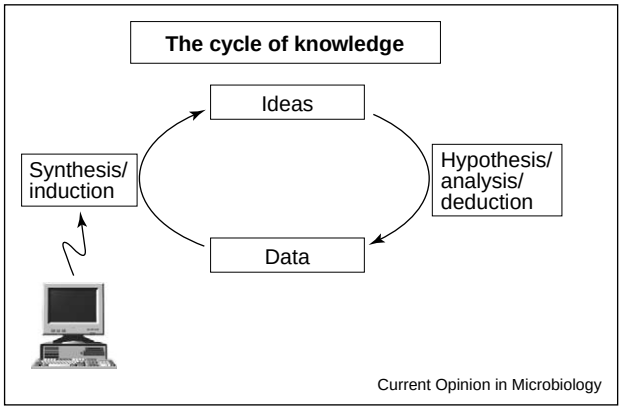
Figure 1



Bibliometric and text mining analysis of the recent metabolomics literature (to the end of 2003). **(a)** The growth in metabolomics papers judged by searching titles and abstracts of Web of Knowledge using 'metabolom'* as the search term. **(b)** Text mining analysis of the metabolomics literature. The text mining tool Ref Viz (Adept Scientific) was used to cluster, according to their keywords, papers that had 'metabolome' or 'metabolomics' in their title. The main groupings seem to be based on whether the emphasis is on technologies, on integration with other omics, or in predicting higher order properties such as disease.

without chromatographic separation. Although comparatively expensive, these sophisticated instruments will have a significant role in the development of metabolomics, especially when problems with ion–ion interferences at high concentrations have been overcome. While their sensitivity and resolution mean that they can be prone to artefacts (gratuitous interferents), there is a clear implication from a series of metabolomic studies that we have only just begun to recognise how many metabolites a typical cell can contain or produce [9••]. For instance, the pioneering studies by Fiehn and colleagues of *Arabidopsis thaliana* [19] indicated the presence of some 326 metabolites; better deconvolution of the data has raised this to over 1000 (Fiehn, personal communication). Thus multidimensional separation methods are coming to the fore [20•] as they can routinely separate more than 1000 compounds [21,22].

Figure 2



Scientific advance may be seen as an iterative cycle linking knowledge/ideas/mental constructs ('ideas') and observations/data ('data'). The hypothetico-deductive mode of reasoning uses background knowledge to construct a hypothesis that is tested experimentally to produce observations. This is only a part of the story, however, as the inductive and abductive modes of reasoning are purely data-driven, i.e. are based purely on generalising rules (or ideas or hypotheses) from examples (and the hypothesis is the end, not the beginning). Because of the high dimensionality of typical data, computer-intensive methods are required to turn the data into knowledge. Scientific advances should (and often do) exploit both deductive and inductive modes of reasoning in an iterative cycle [2,5••].

Liquid-chromatography-mass spectrometry (LC-MS) methods typically have somewhat lower chromatographic resolution than do GC-MS methods, but can access much greater mass ranges because volatilisation (and hence derivatisation) for the chromatographic step is unnecessary. Liquid-phase methods such as LC-MS can be subject to matrix effects (numerous causes often being bundled together under the somewhat unhelpful and inaccurate catch-all term 'ion suppression'), a major one being the presence of non-volatiles, which may reduce the evaporation of volatile ions during the electrospray process [23]. Although reverse phase methods are widely used, normal phase methods can be highly useful in the separation of more polar compounds. However, ion-exchange methods require the use of salts,

which can interfere with mass spectrometry methods, and Tolstikov, Fiehn and colleagues [24,25•] have developed an excellent new hydrophilic interaction liquid chromatography (HILIC) method for this, coupled to electrospray mass spectrometry. In the liquid phase, capillary electrophoresis coupled to mass spectrometry has been used to separate a variety of anionic metabolites from *Bacillus subtilis* [26•], many of which could be quantified accurately by mass spectrometry. Coulometry is another sensitive and somewhat underutilised detection method; Matson and colleagues give an example in which some 600 metabolites could be discriminated [27].

A new development involves the direct injection of the sample into a low-resolution electrospray mass spectrometer [28,29,30••], where quality data are obtainable in less than 1 min, opening up the use of such methods in high-information, high-throughput screening. Matrix-assisted laser desorption ionisation (MALDI) mass spectrometry — the other soft-ionisation mass spectrometric method of choice in proteomics — uses a matrix, typically of aromatic acids, whose molecular weight is similar to those of typical metabolites and thus disallows the mass spectrometric measurement of the latter. Siuzdak and colleagues have neatly avoided this problem via the development of the matrix-free 'desorption ionisation on silicon' (DIOS) method [31]. They have recently shown that when interfaced with a tandem time-of-flight mass spectrometer it provides an extremely convenient tool for metabolite detection [32•], and that the quantitative reproducibility of the method is significantly enhanced by using electrospray deposition [33]. Mass spectrometer conditions are normally adjusted rather arbitrarily, but the search space of possible conditions is huge. The use of a genetic search algorithm to improve substantially the quality of mass spectra from complex mixtures showed both that excellent improvements could be obtained in a comparatively short time and that matrix effects could be reduced or eliminated by varying the mass spectral conditions systematically [34•].

Although it will probably never get close to matching mass spectrometry for sensitivity, NMR continues to improve in resolution and sensitivity [14•,35–37], and has uncovered novel inborn errors of metabolism [38]. Its chief virtue is arguably its non-invasive nature, which can allow one to obtain spatially resolved metabolic profiles and to investigate metabolomics *in vivo* [39], while the nominal independence of the magnitude of the response from different non-exchangeable protons is helpful. For historical reasons it is more commonly applied than mass spectrometry to mammalian samples, and some large-scale studies show reasonably good inter-laboratory reproducibility [40]. Additional specificity can be obtained by using fluorinated metabolites (e.g. [41]). LC-NMR and LC-NMR-MS are newly (re)emerging techniques, reviewed briefly in [42,43] and extensively in [44].

Like NMR, vibrational spectroscopies such as Raman and Fourier transform infrared (FTIR) spectrometry are comparatively insensitive, but the latter especially allows high-throughput screening and classification of biological samples [45], and equally fits the 'omics philosophy' of providing unbiased, whole-system measurements. Among recent examples is its use in detecting the microbial spoilage of meat [46•], where the onset of proteolysis could be clearly identified. FTIR is also a valuable method for the high-throughput screening of mutant strains for different levels of target metabolites [47], a typical recent example being a study of plant cell wall mutants [48].

For fundamental reasons outlined in the theory of metabolic control analysis (MCA), changes in individual enzyme (or presumably transcript) levels have little effect on fluxes but major effects on metabolite concentrations (e.g. [8•,49,50]). Following on from this, where the transcriptome and metabolome have been compared on the same samples, the metabolome indeed seems to be more discriminatory [51].

Finally, we note that most metabolome measurements are 'metabolic snaphots' [49] (and see http://dbk.ch.umist.ac.uk/WhitePapers/mcabio.htm), and what is really desired are methods for reporting, non-invasively and without modifying them, changes in metabolite concentrations in living cells in real time, for which optical strategies are likely to be required. As well as purely spectroscopic methods (UV/Vis, Raman, infrared), molecular biological methods can provide *in situ* sensors, an excellent example being that of the work of Fehr *et al.* [52•].

Making sense of raw metabolomic data

Deconvoluting raw metabolomic data can mean at least three things: (i) working out the signal from metabolites that are imperfectly separated using a 'hyphenated' chromatographic method such as GC-MS, and hence their concentration, (ii) providing a chemical identity for metabolites reproducibly recognised as being present as judged by for example their retention index and mass spectrum, and (iii) using the metabolomic data to reconstruct the metabolic networks in which they participate. (For reasons of space, and although it is an important area, the use of chemometric methods for manipulating the very high-dimensional metabolomic data so as to classify samples according to some scheme of interest (e.g. [53]), is not considered in any detail here.) The first is very important, and improved (and preferably public, non-hidden, non-proprietary) algorithms are required here, with easy-to-use interfaces and high but controllable degrees of automation that process the raw 'hyphenated' data as inputs and produce lists of metabolites as the output. Sumner and colleagues [54••] have produced MSFACTS (metabolomics

spectral formatting, alignment and conversion tools) for this purpose. A high-speed peak alignment algorithm was devised by Synovec's group [55], while an interesting paper by Brereton and colleagues compared a series of methods commonly used for identifying the number of substances in a complex peak [56•], an important prerequisite to optimal deconvolution.

The second of these ('spectrum-to-structure') has not been adequately attacked, and needs automating, since in plants some 80% of metabolites recognised by mass spectrometry have mass spectra that do not appear in the standard libraries, which have concentrated more on organic chemical than on natural biological metabolites. Curiously, one of the earliest forays into computational machine learning, the DENDRAL and METADENDRAL projects [57–59] sought explicitly to enquire as to whether this problem could be automated, but comparatively little in the mass spectral world has happened since. While the picture for NMR data is somewhat rosier [60,61,62•,63,64], this 'spectrum-to-structure' problem remains an important component of 'making sense of the soup', as it is hard to argue that we understand a metabolic system when we do not even know what most of its metabolites are.

The third of these 'deconvolution' issues, metabolic network reconstruction, is dealt with below.

However, another important feature of post-genomics data is represented by their storage, along with the relevant metadata, in suitable databases constructed according to a public and standardised data model [6•,7•,65•], as has already happened for transcriptomic [66,67] and proteomic data [68•,69].

Developments in metabolic modelling

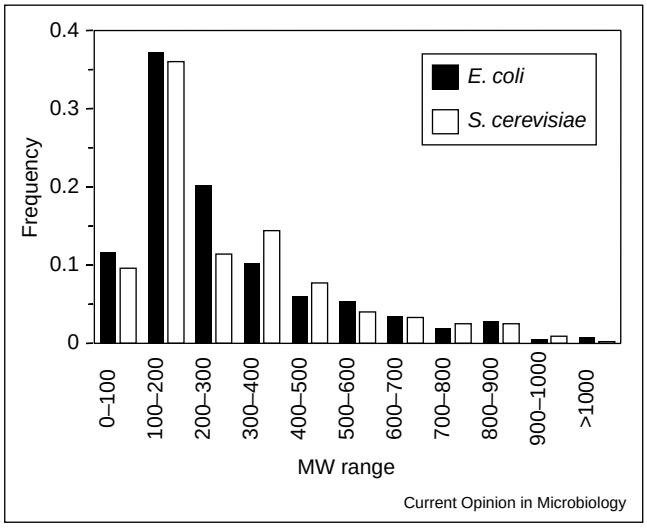
Although we ultimately need to solve the inverse problem [70], metabolic modelling in the 'forward' direction, typically using linked ODE solvers such as *Gepasi* [71] (and see links to others at http://dbk.ch.umist.ac.uk/sysbio.htm#links), continues to provide important knowledge. An especially useful development is the production of data standards, especially the Systems Biology Markup Language (SBML http://www.sbml.org) [72••], which will allow interoperability between different models, including their metadata, and modelling packages. Regarding the latter, the well-known E-cell system [73] has now been revamped and extended to work not just under Linux but also on several operating systems including MS-Windows [74•] (and see http://www.e-cell.org/), which will undoubtedly extend its user base substantially. The FluxAnalyzer [75•] is a MATLAB-based system for the modelling of flux distributions in metabolic pathways, while advances in the important area of metabolome visualization include GenMAPP [76].

Metabolic network organisation and reconstruction

Recognising that metabolites and metabolomes are system variables, the problem of reconstructing metabolic networks, which themselves constitute part of the parameters of the system, becomes an 'inverse problem' or one of system identification [12•,77,78,79••,80•]. Several general questions immediately arise about the size, nature, structure and organisation of metabolic networks [81••]. As to the ostensible size of the 'natural' metabolome, we should comment that many organisms can and will metabolise xenobiotics for non-nutritional purposes, making the potential size of the metabolome practically infinite, and it is possible to read values such as 200 000 for the claimed number of (mainly secondary) metabolites in the plant kingdom [13•]. However, annotated genomic data alone can provide the baseline of reactions, which are more or less well known or may be presumed to occur under at least some conditions in the organism of interest. Although these are available in the general metabolic databases such as KEGG (Kyoto encyclopaedia of genes and genomes; http://www.genome.ad.jp/kegg/) [82], it is necessary to produce organism-specific ones. Important steps in these directions include the Ecocyc project [83,84] (note license conditions) (see http://biocyc.org:1555/ECOLI/class-subs-instances?object=Pathways) and the related MetaCyc sites (http://metacyc.org/), while the latest *E. coli* model has 931 unique biochemical reactions [85•,86•]. The yeast metabolic reaction scheme [87••] is especially useful, as it also exploits biochemical and physiological knowledge (and not merely reconstructions based solely on genomic data), sequence matching and reaction guessing. These models give numbers of metabolites in the hundreds, which are comparatively easy to handle, and while these will be underestimates due to (i) imperfect knowledge, (ii) the lack of specificity among enzymes, and (iii) the production of substances at very low concentrations that we do not routinely detect, they form a very good starting point. An analysis (Figure 3) of the distribution of molecular weights (MWs) of the metabolomes of *E. coli* [88••] and *S. cerevisiae* [87••] suggests that most of the major metabolites are under MW 300. A candidate mouse metabolome is already available [89], and one for humans must be imminent. A very nice example of the use of post-genomic metabolic reconstruction concerns the bacterium *Tropheryma whipplei*, the causative agent of Whipple's disease, whose presence was originally detectable only by molecular methods of nucleic acid hybridisation. Until recently the organism could be grown only in fibroblasts. However, an analysis of its genome showed that it lacked the ability to produce several amino acids, which allowed Renesto *et al.* [90] to design a medium that would support its growth axenically.

Solving inverse problems of metabolic network reconstruction from metabolome data (even with a well-observed time series) in general is hard, but there are

Figure 3



Histogram of molecular weights of typical microbial metabolites. *E. coli* data are from [88••] and were kindly supplied by Dr Irilenia Nobeli (who points out that some of the larger molecular weight metabolites such as lipids are not included as they were not then in Ecocyc). *S. cerevisiae* data were extracted from [87••]. Although the assumptions are rather different it is clear that most of the common microbial metabolites are of relatively low molecular weight.

several constraints that can help to make it easier, and here real progress is being made. First, several important studies suggest that metabolic networks often show a scale-free [81••,91•,92•] or 'small world' [93] kind of organisation. Second, there are important stoichiometric constraints and 'elementary modes' [94,95,96•,97••,98••] that restrict both the networks and their regulations which are possible, although further restrictions are necessary to make even these analyses manageable [99•]. As with blood vessels in higher organisms, the metabolic fluxes in *E. coli* are arranged into a major 'backbone' that dominates the main fluxes [100••]. However, we note that many of these representations use graph theory, which is somewhat 'static', and may fail to capture the richness of the dynamics and control of such networks [101•].

This said, a particularly elegant strategy combines the networks that are reconstructed qualitatively from the genomic data with the constraints imposed by (quantitative) mass balancing [102,103••]. This allows one, subject to additional constraints about how cells 'choose' or have evolved to regulate their fluxes in terms of growth rate or growth yield (most likely the former [104]), to make some extremely powerful and successful predictions of whole-cell behaviour at the metabolic and physiological levels from such *in silico* analysis alone [105••,106,107•,108••, 109,110••]. The success of this endeavour highlights the very great importance of the topological structure of metabolic networks (independently of their kinetic properties), to their effective functioning.

An especially helpful analysis of metabolic networks in terms of 'network motifs' was introduced by Alon and colleagues [111••,112••] (and see also much work over the years more specifically on the design of metabolic and, especially, genetic networks by Savageau and colleagues, for example [113,114•]). Network motifs, by loose analogy with protein structural motifs, are arrangements of reactions, including feedback structures, which regularly occur in biology and are therefore assumed to have functional use [115•]. Codifying them is thus a very important activity. Of all the possible feedback arrangements between n separate elements, a very restricted subset is found to occur regularly in nature (i.e. to have been selected by evolution). One example is the series of feed-forward loop network motifs [116•]. As well as those bestowed by nature, it is possible to produce artificial regulatory networks. To this end, Guet *et al.* [117] used combinatorial methods to vary the 'logical phenotypes' exhibited by various strains of *E. coli*, while Pilpel and colleagues did the same for regulatory networks [118].

A development of MCA called co-response analysis [119] has proved of value both in pattern recognition analysis of the metabolome [120] and in recognising that the co-variation of pairs of metabolites from the same organism under different conditions can provide very useful information of their 'connectedness'. Thus Kose *et al.* [121], drawing on elements of graph theory, developed clique correlation analysis, and especially the use of maximal cliques, for the visualisation of metabolomic data. More recently, Steuer *et al.* [122•] have sought to relate metabolite covariance matrices, while statistical and machine learning methods have proved useful — as with transcriptomics and proteomics data — in deconstructing the highly multivariate data that metabolomics provides [123•]. In an analysis of *E. coli* mutants, a genetic algorithm analysis of direct injection mass spectra identified just two or three peaks that served to pinpoint the nature of the mutation involved [124]. Indeed in our hands genetic algorithms and genetic programming have proved extremely successful strategies for discovering which metabolites are most important for 'explaining' some biological process of interest (e.g. [3•,30••,46•125–128,129•]). In many cases we simply do not know the kinetic properties of the system's components, and genetic programming has also proved useful in the analysis of the inverse problem of metabolism referred to above [130••]. Another very useful strategy is to approximate the kinetic properties with generic rate equations, for example by power laws [113], while Heijnen and colleagues (e.g. [131•] have more recently exploited lin-log kinetic equations for this purpose.

Perturbing, rather than merely observing, metabolic networks is much closer to the (not so) new 'systems' biology that is largely aimed at solving the inverse problem of genetic and metabolic networks. However, deciding

which perturbation to make is a very interesting area (known as 'active learning' [132,133]), as this can greatly improve the quantity and quality of information available from a particular experiment [5••]. Vance *et al.* [134•] presented a computational strategy for working out the causal connectivities of metabolic networks from such perturbations, and a somewhat related strategy was used by Kholodenko and colleagues for genetic and signalling networks [135•]. This, and a slightly different strategy by de la Fuente, Mendes and colleagues [136•] (which also used linear perturbation theories as in MCA) are summarised in a readable way by Stark, Callard and Hubank [101•], while Ross gives a differently focused overview of recent advances in deconstructing chemical networks from time series data [137•]. In a similar vein, the site of interaction of mitomycin C in a small subnetwork of the *E. coli* SOS system was identified [138•].

Integrating metabolomics into systems biology

Although such ideas are very far from being new [139,140], there is a huge interest in understanding complex biological systems from the more holistic 'systems' point of view. Such strategies — contrasted with those of the qualitative and more reductionsist molecular biology era [1••,141] — are usually considered to involve both quantitative experimentation and mathematical simulation/modelling (see above) in an iterative fashion [13•,78,142,143]. Although metabolomics measurements have a major role to play in metabolic network reconstruction, true systems biology will require the integration of metabolomic measurements with measurements of the time-dependent concentrations of other types of components. The availability of protein microarrays consisting of all the cloned proteins of an organism will allow them to be screened systematically for drug or metabolite binding [144,145], and this will be a major contribution to integrative biology. A particularly exciting possibility is that of purifying proteins directly from target organisms in a mass spectrometer without cloning them, and thereby producing microarrays directly [146].

Some applications

While the metabolomic data constitute the 'ground substance' for inferring knowledge, and are the focus of this microbiologically oriented review, several very significant findings of basic or applied biomedical interest have emerged from recent metabolomic studies. A major driver is discovering biomarkers (for definition see [147]) or disease status [9••,148,149•]. Thus Nicholson, Grainger and colleagues [150•] used ^{1}H-NMR measurements together with discriminant partial least squares analysis to distinguish various forms of coronary heart disease via blood samples, although only a small number of samples was used. As well as assessing biomarkers for clinical diseases, it is also vital to gain an understanding of the normal human serum metabolome in health [151], where interesting diet-dependent changes could be observed [152].

There is much interest in determining the mode or site of action of compounds, in functional genomics, in target discovery and in toxicity assessment, which are thereby interrelated. Ott and colleagues [153,154•] combined NMR profiles and neural networks with great success in detecting the mode of action of crop protection substances in aqueous extracts of plants, while Nicholson and colleagues have been particularly active in developing the use of metabolite measurements for assessing drug toxicity [10•]. It is obvious that these methods are generic, and that metabolomics will increase in importance in toxicology, mode of action analysis and functional genomics.

Many new signals are waiting to be discovered via the metabolomics approach. Shi *et al.* [155•] used a generalised purification and metabolomics approach to detect a novel signalling metabolite, uric acid, released from dying mammalian cells.

On the biotechnological front, Askenazi and colleagues [156] integrated transcriptome and (limited) metabolome profiles, the latter measured using LC-MS, to improve the yields of lovastatin and (+)-geodin from *Aspergillus terreus*, and it is clear that improved understanding of the metabolic pathways and fluxes [157] to products of biotechnological interest should be revolutionised by the exploitation of the types of metabolomics and network biology methods reviewed here.

Future perspective and concluding remarks

Metabolomics is a burgeoning science (Figure 1) that brings together analytical technology, genomics and computation, and lies at the core of the systems biology agenda. Major areas for development will involve improving the sensitivity, universality and discrimination of our instruments, and this will involve new approaches and better deconvolution. Structural (i.e. chemical) identification of the many uncharacterised metabolites is still a very important and routinely unsolved problem, while integrating metabolic models in genomically characterised organisms with their experimentally determined metabolomes will allow an iterative improvement of our understanding of the latter. This can be seen as the hallmark and purpose of the systems biology agenda.

Acknowledgements

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Lazebnik Y: **Can a biologist fix a radio? - or, what I learned while** •• **studying apoptosis**. *Cancer Cell* 2002, **2**:179-182.
A wonderful and amusing piece extolling the virtues of a top-down and quantitative approach to understanding complex biological networks. The radio engineer succeeds because s/he has a wiring diagram, not merely a list of isolated components that may be present but without any knowledge of their values and what they interact with.

2. Kell DB, Oliver SG: **Here is the evidence, now what is the hypothesis? The complementary roles of inductive and hypothesis-driven science in the post-genomic era**. *Bioessays* 2004, **26**:99-105.

3. Kell DB: **Genotype:phenotype mapping: genes as computer** • **programs**. *Trends Genet* 2002, **18**:555-559.
Genetic programming methods can provide a straightforward link between the genotype and for example metabolomic phenotype of an organism, which maybe useful in both genetic and other nonlinear mappings. The strategy could clearly be used to determine important connectivities in nonlinear metabolic networks.

4. Sweetlove LJ, Last RL, Fernie AR: **Predictive metabolic engineering: a goal for systems biology**. *Plant Physiol* 2003, **132**:420-425.

5. King RD, Whelan KE, Jones FM, Reiser PGK, Bryant CH, •• Muggleton SH, Kell DB, Oliver SG: **Functional genomic hypothesis generation and experimentation by a robot scientist**. *Nature* 2004, **427**:247-252.
The authors implemented an entirely closed loop system of automated experimentation in which a computer containing background knowledge proposed the 'best' (most discriminating) experiment, performed it and evaluated it *seriatim* before performing the next cycle, without human intervention. The method was applied to the identification of mutants in aromatic amino acid biosynthesis in baker's yeast, using growth and nutrient feeding experiments. The strategy could be applied to many areas of post-genomic biology and elsewhere.

6. Mendes P: **Emerging bioinformatics for the metabolome**. • *Brief Bioinform* 2002, **3**:134-145.
Metabolome data differ from transcriptome and proteome data and have special bioinformatics needs, which are reviewed here. Special emphasis is placed on the important issue of data visualisation.

7. Sumner LW, Mendes P, Dixon RA: **Plant metabolomics:** • **large-scale phytochemistry in the functional genomics era**. *Phytochemistry* 2003, **62**:817-836.
A first-class and easy-to-read review of the technology and science of metabolomics, concentrating on plants, and including a helpful discussion of database issues.

8. Fiehn O: **Metabolomics: the link between genotypes and** • **phenotypes**. *Plant Mol Biol* 2002, **48**:155-171.
A very nice review, including a detailed discussion of the differences between metabolomics, metabolic profiling and metabolic fingerprinting and methods for performing them. GC-MS remains the most common and 'gold standard' method, but other methods are being introduced.

9. Harrigan GG, Goodacre R (Ed): *Metabolic profiling: its role in* •• *biomarker discovery and gene function analysis* Boston: Kluwer Academic Publishers; 2003.
A useful compendium of articles from many of the main players in metabolomics, covering many issues in more detail than is possible (or likely) in a single review. The cover, which is based on an iceberg, illustrates the inevitable tendency to believe that we have measured everything when we have merely measured all that we, or our chemometrics, can see.

10. Nicholson JK, Connelly J, Lindon JC, Holmes E: **Metabonomics:** • **a platform for studying drug toxicity and gene function**. *Nat Rev Drug Discov* 2002, **1**:153-161.
A review of metabonomics (metabolomics) with specific reference to its use in assessing mechanisms of toxicity from the metabolic profiles in urine. A particularly useful feature of this review is the helpful figure relating specific markers in the profiles to the major target sites.

11. Watkins SM, German JB: **Metabolomics and biochemical profiling in drug discovery and development**. *Curr Opin Mol Ther* 2002, **4**:224-228.

12. Fiehn O, Weckwerth W: **Deciphering metabolic networks**. • *Eur J Biochem* 2003, **270**:579-588.
A review that aims to concentrate on methods aimed at exploiting metabolomic measurements for deciphering metabolic networks, but also covering many of the existing or emerging methods for measuring the metabolome itself.

13. Weckwerth W: **Metabolomics in systems biology**. *Annu Rev* • *Plant Biol* 2003, **54**:669-689.
A review of metabolomics concentrating on its contribution to the emerging field of systems biology.

14. Lindon JC, Holmes E, Nicholson JK: **So what's the deal with** • **metabonomics? Metabonomics measures the fingerprint of biochemical perturbations caused by disease, drugs, and toxins**. *Anal Chem* 2003, **75**:384A-391A.
A review of the 'metabonomics' agenda, concentrating on high-resolution NMR and pattern recognition, although somewhat misrepresenting the origins and usage of the term 'metabolomics'. Stress is laid on the use of automation in making such methods rapid and robust, which means the cost-per-sample can be low even when using rather expensive equipment.

15. Nicholson JK, Wilson ID: **Understanding 'global' systems** •• **biology: Metabonomics and the continuum of metabolism**. *Nat Rev Drug Discov* 2003, **2**:668-676.
A review that stresses the relationship between diet, the gut microflora, and the host's genome, metabolome and disease. It includes a figure illustrating 'metabolic space' with over 5000 samples and provides a clear statement of the authors' distinction between the metabolome and the 'metabonome', the latter of which is said to relate to the metabolic responses to pathophysiological stimuli or genetic modification.

16. Goodacre R, Vaidyanathan S, Dunn WB, Harrigan GG, Kell DB: • **Metabolomics by numbers: acquiring and understanding global metabolite data**. *Trends Biotechnol*. 2004: in press.
An introductory overview to the field of metabolomics that lays particular stress on handling the data generated.

17. Marshall AG, Hendrickson CL: **Fourier transform ion cyclotron resonance detection: principles and experimental configurations**. *Int J Mass Spectrom* 2002, **215**:59-75.

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110. Burgard AP, Pharkya P, Maranas CD: **OptKnock: A bilevel**
•• **programming framework for identifying gene knockout strategies for microbial strain optimization**. *Biotechnol Bioeng* 2003, **84**:647-657.
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111. Milo R, Shen-Orr S, Itzkovitz S, Kashtan N, Chklovskii D, Alon U:
•• **Network motifs: simple building blocks of complex networks**. *Science* 2002, **298**:824-827.
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112. Shen-Orr SS, Milo R, Mangan S, Alon U: **Network motifs in**
•• **the transcriptional regulation network of *Escherichia coli***. *Nat Genet* 2002, **31**:64-68.
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