of this bacterium[30]. This kind of dual effect of PIs could prove effective in various host–pest and host–pathogen systems.

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Meeting report

Making cells work – metabolic engineering for everyone

Recombinant DNA technology has evolved to the point where it is now feasible for most laboratories to apply it. Apart from its uses in genetics research, this technology has opened up the possibility of engineering metabolism for biotechnological ends, typically in metabolite overproduction[1]. Not only can one overexpress genes of a particular organism, one can also introduce foreign genes to redesign metabolism. Recently, the Engineering Foundation organized a conference on this increasingly popular subject*. Participants were well balanced between academia and industry and came from many countries (roughly half from the organizing country, USA). The topics covered were diverse, ranging from applications of metabolic engineering (ME) to microbial, plant and animal metabolism, to the manufacturing of chiral compounds, with two sessions on tools (experimental and theoretical, and computational). Two workshops took place to debate optimal host cell systems and to define the field of ME. Two poster sessions complemented the subjects of the lectures.

In the keynote lecture, Gerald Fink (Whitehead Institute, Cambridge, MA, USA) sketched what he believes will be the biology of the post-genomics era, which we are entering with the completion of the sequences of the genomes of many organisms. Using examples from the fungal world, he stated the importance of identifying the different behaviour modes, or algorithms, with which organisms perform their functions. We can learn a lot both from the similarities of the algorithms of related organisms and from their differences, but for this we need to identify and understand the expression of the large number of open reading frames whose function is as yet unknown. (Boris Magasanik brilliantly exemplified how complex this will be by describing the regulation of nitrogen-limited growth of *Escherichia coli* and *Saccharomyces* in a memorable after-dinner lecture.) For example, all known fungal pathogens have to switch to a filamentous form before invading the host, and even our old friend *Saccharomyces* is now known to switch to a filamentous mode at some stages of its life cycle. The conditions that influence this switch of 'growth algorithms' could be of major importance for the brewing industry in the control of flocculation of their cultures, and also for the pharmaceuticals industry in the design of drugs to prevent infection by fungal parasites. In the post-genome era the challenge is to identify the function of all sequenced genes and, more importantly, to understand their concerted expression (the phenotype). Other speakers showed specific examples of algorithms that have been decoded and that are now being manipulated to obtain new products (some argued that the word engineering is more relevant to the design of new functions than the optimization of existing ones). The production of antibiotics with aromatic rings by *Streptomyces* can be described by four basic processes (some of which are iterated) (Richard Hutchinson; University of Wisconsin, WI, USA): (1) choose (between substrates); (2) count; (3)

*The meeting 'Recombinant DNA Biotechnology: Focus on Metabolic Engineering' was organized by the Engineering Foundation, and was held in Danvers, MA, USA, 6–11 October 1996.

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fold; and (4) cyclize. Novel products have been produced by the combination of various polyketide synthases from different organisms, or even simply by changing the order of their genes in the genome. The understanding of the glycosylation processes in eukaryotes has advanced greatly with the help of techniques such as mass spectrometry and NMR and can also be described by 'algorithms' that can be manipulated by the expression of the appropriate genes (e.g. sialyltransferases), and their physiological manipulation (Nigel Jenkins; De Montfort University, Leicester, UK).

As one would expect in such a conference, many engineered 'high producer' systems were described. The applications ranged widely, from the production of ethanol from office waste paper by engineered *E. coli* with genes from *Klebsiella oxytoca* (Lonnie Ingram; University of Florida, FL, USA) to the production of semisynthetic opiates, also by *E. coli* (Neil Bruce; University of Cambridge, Cambridge, UK). A workshop was organized by Scott Power (Genencor International, Palo Alto, CA, USA) to discuss the selection of an optimal host cell for ME. This was centred on the possibility of constructing a basic minimal host cell that would then be a choice platform for adding metabolic modules (a kind of biological Lego® system) to become a high producer for the product of interest. Even though nobody disagreed with the advantages of such a host (simple, well understood), its construction still seems to be far-off, mainly because our lack of knowledge of the function and, especially, the interaction of most gene products (which tend to interact synergistically), means we cannot decide what to delete from the genome. Even leaving patenting issues aside, it seems that a diverse range of hosts will continue to be used for the foreseeable future.

At first sight, and following what is described in most biochemistry textbooks, one might imagine that to increase a metabolic flux we need only identify the rate-limiting enzyme of the pathway and over express it. However, with the development of metabolic control analysis (MCA) it has become evident that the control of flux is distributed throughout a metabolic pathway (David Fell; Oxford Brookes University, Oxford, UK). Peter Jensen (Technical University, Copenhagen, Denmark) described how MCA can deal with a hierarchical process like gene expression and presented a novel experimental tool that has great potential for allowing the measurement of MCA coefficients. This technique is based on the variable induction of artificial promoters. Promoters of different activities (covering a very wide range) can be selected from a library and placed upstream of the gene of interest, thus fine-tuning its expression (as is needed for the accurate determination of control coefficients). This has the additional advantage that once the optimal promoter activity has been determined, the strain is, in principle, ready for use in the industrial process. MCA was able to help in the selection of targets for overexpression for improving starch accumulation in potato tubers (Michael Burrel; Advanced Technologies, Cambridge, UK). A key point in this work was that no significant effects were observed when only one enzyme (the one with initially the highest control coefficient) was overexpressed. When the concentrations of two enzymes were manipulated, the effect was then satisfactory. This theme recurred throughout the conference. Simulations of the aromatic amino acid production pathway point to the same conclusion, and the best targets for the manipulation are those enzymes that have a high flux-control coefficient and also a low concentration-control coefficient so as to affect the concentration of intermediary metabolites minimally (Greg Stephanopoulos; MIT, Cambridge, MA, USA). Many posters also presented results that revealed large effects on flux only when more than one enzyme was overexpressed.

The message from MCA has been received with open arms by this field, though we are still waiting for the recognition given by textbooks. Other theoretical frameworks are also useful for ME, especially the so-called 'flux analysis', which is mainly a stoichiometric analysis, revealing structural properties of the pathway. This representation can be subject to optimization methods (Vassily Hatzimanikatis; ETH, Zurich, Switzerland), which allows the screening of large sets of possible behaviours. 'Flux analysis' has the advantage that one needs to know only the stoichiometry of the pathway of interest, though even that might not be trivial to determine. ^{13}C NMR determination of fractional isotope enrichment of metabolites can resolve the net- and exchange-fluxes of *Corynebacterium glutamicum* towards L-lysine production (Lothar Eggeling; Forschungszentrum Jülich, Jülich, Germany). Peter Vanrolleghem (University of Gent, Gent, Belgium) described a structured approach to estimate the unknown stoichiometric coefficients. The temporal aspects of steady-state metabolism (mainly the relaxation times after perturbations) should also be taken into consideration for rational improvements (Bernhard Palsson; University of California, San Diego, CA, USA). The combination of theoretical analysis frameworks with experimental determinations of fluxes and metabolite concentrations is the only way to provide a rational means for ME (Jens Nielsen; Technical University, Denmark). With regards to the experimental measurements, many advances will be achieved as novel multivariate, non-invasive methods of analysis become more widely available. On the theoretical side, user-friendly computational tools that perform the required calculations (especially in these days of cheap computational power) are becoming available (Pedro Mendes; University of Wales, Aberystwyth, UK), and will open many new doors to our understanding of metabolism and, therefore, to the engineering of new pathways.

The industrial application of ME is, unfortunately, determined not only by the availability of high-yielding producer strains. Often the overall costs of biological fermentations are higher than those of chemical synthesis, not least for reasons connected with purification costs (Paul Reider; Merck Research Laboratories, Rahway, NJ, USA). The challenge is to devise methods that produce high chemical and enantiomeric purities that are practical on a large-scale, safe, environmentally sound (and this is often where biotechnology is at an advantage over chemical synthesis), efficient and economical. The future of ME lies ultimately in its answers to these problems, rather than just on the characteristics of the actual engineered strains.

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