The cell factory needs a model of a factory

Letter

A recent conference on metabolic engineering* showed the progress in the various fields of modelling metabolic processes. It also revealed that, in this era with a huge amount of genomic and proteomic data, research on the related (microbial) physiology lags behind and needs special attention. There are many worrying questions that need answers. Why is the physiological knowledge abstracted as metabolic modelling still way behind the known facts? Does the metabolic modelling of cellular metabolism limit the speed of research in the long term[1]? Can and do we learn from metabolic models? Do we need other expertise such as control engineers for studies on metabolic control?

In the European Framework programmes, metabolic studies on microbial cells are organized under the theme *Cell factory*, implying that we see the cell as a factory to make useful things. However, in the *Cell factory* programme, the comparison between a factory and a cell is limited to that idea only. What else could we learn from this comparison?

The cell can also be compared to a factory or a small company operating in a competitive market. Knowing the total inventory of the factory (here, all the enzymes and other proteins) does not indicate how it works, what its performance will be or how to improve it. Many similar machines with slightly different properties can be found in the various inventories. Similarly, during the evolution of cells, the proteins and various cell structures have been optimized to function under the conditions that the cell is faced with. In the case of man-made machinery, there is a driving force towards more-efficient equipment and optimal processing. At the factory level, this leads to quality control, quality assurance and Total Quality Management. The optimal modes of operation only hold for a given condition or steady state; in a thriving factory, using only one way of working will weaken its position in a changing market with changing supplies. Finding these modes implies management and control of the required change.

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Systems approach

The above analogy can be applied to the way in which we study and attempt to improve metabolic systems. By contrast, there is a different approach (the holistic or systems approach) that is being discussed frequently[2]. The need to study the interaction of all cellular components is top of the list for many biologists but, even though data at the molecular-interaction level are being generated at a considerable rate, there is only a limited increase in our knowledge of how all the components make the system operate.

In vitro and *in vivo* studies have led to the development of models at the molecular and pathway levels, and these models have produced detailed enzyme or pathway kinetic equations[1,3]. The models have been extended to quasi-steady-state metabolic-flux-balance calculations that take into account either the known metabolic network or an abstracted version of it[1,3]. However, the mathematical modelling of related cellular control is still under-developed.

The management of these cellular processes must include the most important property of the factory – its strategy. A cell factory has the ability to carry out various different strategies, depending on various influences: (1) the inputs or direct opportunities, as sensed from the cellular environment (i.e. the raw materials available); (2) the current capabilities and the potential to add or increase capabilities by induction or modulation; (3) the ability to change the cellular machinery to cope with the new desired situation; (4) managing change at the cellular level; and (5) the outputs required to survive, grow and compete. Cells may also make byproducts to hinder competitors or become dormant (sporulate) if the internal or external conditions are not optimal.

Cybernetics

The responses of cells under natural conditions have evolved to the optimal level given a certain ecological niche. The Darwinian principle of the survival of the fittest results in cells that have not one but a series of optimal responses arising from basic strategies. This cybernetic principle was described mathematically in 1948[4]. Here, we define a cybernetic model system as an optimal, self-controlling system with a minimal amount of deterministic knowledge.

The principle of optimal metabolic responses has been known for a long time[6–8]. However, (microbial) physiologists have only recently begun to use the mathematical implementation of cybernetic models and, even then, only to a limited extent. The models take account of a limited number of substrates and conditions. The internal metabolite levels are assumed to be is a steady state. In most of these studies, the metabolic strategy is to maximize the resulting growth rate[7–9], which is the most obvious strategy of a cell. Despite this relatively simple strategy, the performance of these models is remarkably good, taking into account the small number of active parameters involved[9].

Recently, the cybernetic approach has been extended to non-steady-state conditions and to conditions that do not depend greatly on the optimization of growth rate alone. In accordance with the cybernetic principle, no internal kinetic enzyme equations or interactions are assumed. Thus, for a given external and internal metabolic state, the rates of the metabolic network are optimized. This involves estimating from 62 pathway rates or fluxes for a large yeast model (M. L. F. Giuseppin and N. A. W. van Riel, unpublished) down to only seven rates for a model of the central nitrogen metabolism of yeast (N. A. W. van Riel *et al.* unpublished). This optimization of rates is calculated for each small time interval and requires intensive flux optimizations.

These models describing the non-steady states during transient states or substrate pulses reveal more-dominant strategies used by the cells. One such strategy is the control of homeostasis for the cellular components. Here, control-engineering principles have been shown to be very useful. This control of homeostasis is implemented in the model by using proportional integrative (PI) controllers similar to those used in classical

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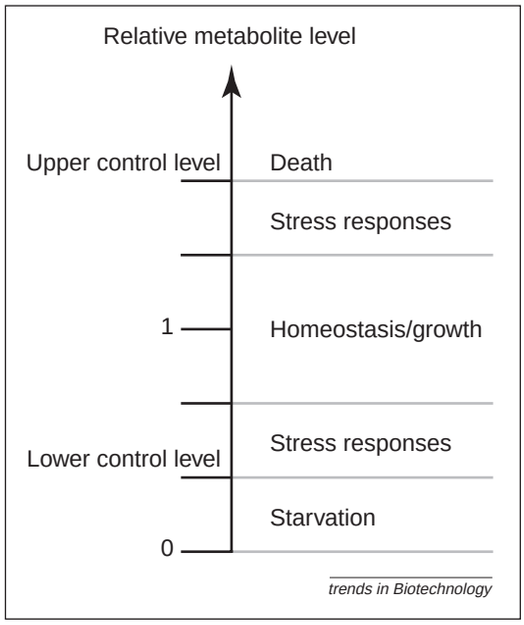

Figure 1

The cellular response strategies used in cybernetic modelling. This graph indicates how the relative levels of cellular compounds (e.g. metabolites) trigger various strategies. The arbitrary control levels indicate the levels at which the cell can control its metabolism to a suitable new state. The optimal state is normalized to a value of 1.

control engineering. The error or deviation from the target state value is used in these controls. The control of each pathway or key component can effectively be described by two parameters of the PI controller (M. L. F. Giuseppin *et al.*, unpublished). The dynamic responses have been studied in nitrogen-limited cultures of *Saccharomyces cerevisiae*, pulsed with various nitrogen sources. Various *S. cerevisiae* mutants in central nitrogen metabolism have been studied in the same way.

The relative importance of substrate uptake versus control of homeostasis was determined and may allow the hierarchy or relative weights of strategies to be identified. This information enabled the model to predict the dynamic metabolic responses of various mutants effectively. Combined with traditional structured models, the cybernetic model can now be used to learn and to design experiments based on mutant behaviour as described by van Riel *et al.*[3] In addition to the hierarchy, a nested structure or sequence of strategies can be identified. For example, if homeostasis is poorly controlled, the depletion of precursors may lead to a reduction in the growth rate, the induction of additional pathways and, eventually, strategies leading to a dormant stage (e.g. sporulation, G_0 stage) or even apoptosis. Figure 1 shows a scheme with different metabolic states and related strategies.

The recent systems or cybernetic approaches need to be studied in more detail by biologists. The study and application of metabolic strategies may be closer to the overall biological behaviour of the living organism than studying its parts in detail. We may urgently need more-appropriate tools to determine the role and subtle effects of the majority of proteins with still-unknown functions. Models that integrate the known parts can be used to validate the putative roles of proteins in the overall cell function. Together with flux-analysis tools such as metabolic-control analysis[10] and metabolic-flux balancing[3,9], cybernetic techniques form a powerful set of tools to determine how exactly a cell works.

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