PERSPECTIVES: BIOCHEMISTRY

How to Make a Superior Cell

Gregory Stephanopoulos and Joanne Kelleher

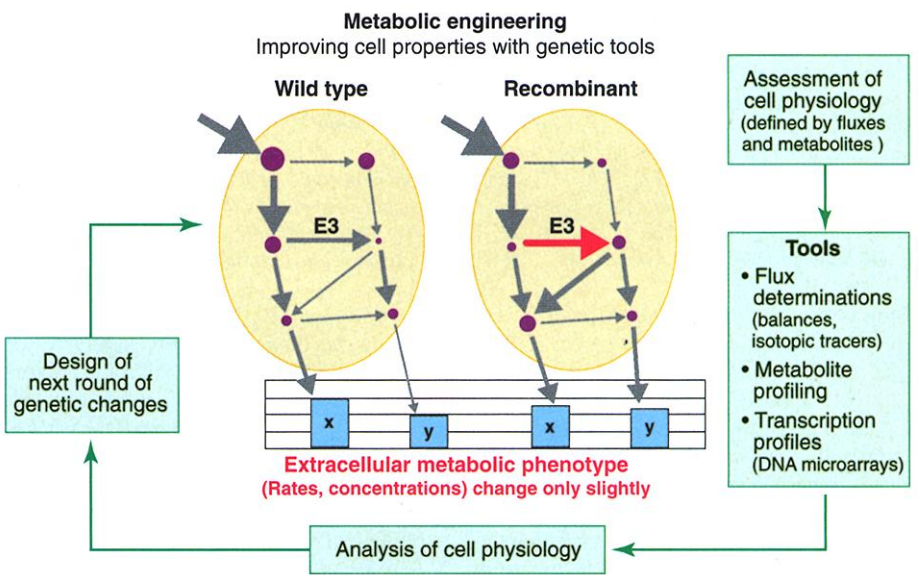
Biologists are experts at improving on nature. By randomly mutating and selecting cells, they have created cellular "minifactories" that churn out molecules of industrial and medical value. With newer genetic tools, cells have been further improved to produce foreign products or modified versions of their own molecules (*1*). Notable successes include *Escherichia coli* bacteria that make indigo (*2*), "golden rice" that synthesizes β-carotene (which may combat vitamin A deficiency in developing countries) (*3*), and the fungus *Streptomyces*, which makes superior polyketide molecules thanks to manipulation of its polyketide synthase enzyme complex (*4*). To this list can now be added the engineering feat of Zaslavskaia and colleagues (*5*), reported on page 2073 of this issue. They have converted an obligate photosynthetic microbe, the diatom *Phaeodactylum tricornutum*, into a heterotrophic organism by introducing a human gene (*glut1*) that encodes the red cell glucose transporter protein. Diatoms normally harness the energy of light through photosynthesis for growth and do not appear to break down simple sugars in the environment (glycolysis) even though they have the enzymes to do so. Yet with the introduction of a single gene, *P. tricornutum* switched its metabolism completely, becoming a heterotroph that metabolizes simple sugars in the absence of light.

The work of Zaslavskaia *et al.* and many others demonstrates that cells can be enticed to make new molecules through introduction of foreign genes. In addition to this strategy, cells producing sufficient quantities of desirable products can be obtained by altering the regulation of genes and the proteins they encode (*6*). For example, yeast can be made to break down galactose by eliminating three negative regulators that inhibit transcription of genes involved in galactose metabolism (*7*). *E. coli* has been engineered to synthesize lycopene through design of a regulatory circuit that ties the expression of lycopene pathway genes to the metabolic state of the bacterium (*8*).

The newest strategy for producing superior cells and microbes is metabolic engineering, which goes beyond gene amplification and the modulation of gene expression. The problem with introducing genes that encode the product of interest into host cells is that gene products interact in intricate, nonlinear, and unpredictable ways so that simple genetic transformations are accompanied by unanticipated and often undesirable side effects. To deal with this complexity, metabolic engineering emphasizes biosynthetic networks in their entirety, addressing questions of metabolic pathway reconstruction, thermodynamic feasibility, the rate of conversion (flux) of metabolites, and control of this flux. This approach represents a shift away from individual enzymatic reactions toward metabolic pathways and bioconversion networks (*9*). This shift is necessitated by the fact that, contrary to widespread belief, kinetic control in metabolic pathways often is not concentrated in a single reaction but is distributed among many reaction steps (see the figure) (*10*). To alter metabolism and to maximize the desirable outcome in bioreaction networks requires intervention at multiple points. Strategies for overproducing microbial metabolites, for correcting mammalian cells with a metabolic defect, or for screening new products to find potential drugs will have a higher probability of success if multiple reaction steps are genetically modified.

Envisage a model biochemical network where improving the characteristics of the cell necessitates the redirection of pathway flux toward metabolite Y rather than metabolite X (see the figure). Clearly, the full impact of a genetic change, such as overexpression of enzyme E3, cannot be fully assessed by the amount of the extracellular metabolites X and Y alone. In this model, as is often observed in vivo, substantial changes in enzyme activity only marginally affect the amounts of such metabolites. A far more informative picture of the resulting cell physiology is obtained by measuring the flux of intracellular metabolites within the network. This information can be used to direct successive rounds of genetic modification. Flux changes in response to genetic modification contain information about the distribution of kinetic control in metabolic networks (*9*). Such flux changes can be readily quantified by combining isotopic labeling of specific molecules in the network with modeling techniques that estimate fluxes from the amount of isotope found in each metabolite.

We predict that metabolic engineering will be applied to the optimization of biosynthetic pathways through coupling rational and combinatorial approaches that, to date, have been largely limited to single-enzyme improvements by gene shuffling and directed evolution (*11*). A particular area of promise is biocatalytic conversion for the production of specialty chemicals, such as chiral pharmaceuticals. In human metabolism, the emphasis on



Complexities in metabolic networks. Wild-type cells are engineered to overexpress enzyme E3 with the goal of increasing the low yield of product Y. However, because of network interactions, overexpression of E3 has a minimal effect on the accumulation rates of either products Y or X. To improve the yield of product Y, multiple steps in the network will have to be targeted and genetically modified. Purple circles indicate pool size of metabolites in the network. Arrow thickness depicts relative flux magnitude of the corresponding reactions.

The authors are in the Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139, USA. J. Kelleher is also in the Department of Physiology and Experimental Medicine, George Washington University School of Medicine and Health Sciences, Washington, DC 20037, USA. E-mail: gregstep@mit.edu

principles underlying metabolic networks provides a template for gene therapy and other strategies to treat metabolic diseases, exploiting principles revealed in simpler, better controlled systems.

An important opportunity is provided by the network features of cellular signaling pathways. Although previously studied in isolation, these pathways are now known to interact with one another (cross-talk): One ligand may regulate the expression of more than one gene, or the expression of a single gene may be affected by more than one ligand (*12*). Technologies—such as DNA microarrays, quantitative proteomics, and improved metabolite measurement methods—that quantify important classes of intracellular variables are proving invaluable for deciphering this cross-talk. The main challenge ahead is the development of computational methods to integrate and make optimal use of such technologies for the elucidation of cell physiology at scales comparable to those now attainable at the gene expression level. Metabolic engineering will be pivotal in this endeavor while continuing its pursuit of cellular property improvement for industrial and medical applications.

References

1. J. E. Bailey, *Science* **252**, 1668 (1991).
2. D. Murdock *et al.*, *Biotechnology* **11**, 381 (1993).
3. X. Ye *et al.*, *Science* **287**, 303 (2000).
4. B. A. Pfeifer *et al.*, *Science* **291**, 1790 (2001).
5. L. A. Zaslavskaia, *Science* **292**, 2073 (2001).
6. J. D. Keasling, *Trends Biotechnol.* **17**, 452 (1999).
7. S. Ostergaard *et al.*, *Nature Biotechnol.* **18**, 1283 (2000).
8. W. R. Farmer, J. C. Liao, *Nature Biotechnol.* **18**, 533 (2000).
9. G. Stephanopoulos, A. Aristidou, J. Nielsen, *Metabolic Engineering: Principles and Methodologies* (Academic Press, San Diego, CA, 1998).
10. D. A. Fell, *Understanding the Control of Metabolism* (Portland, London, 1997).
11. J. A. Kolkman, W. P. Stemmer, *Nature Biotechnol.* **19**, 423 (2001).
12. M. A. Schwartz, V. Baron, *Curr. Opin. Cell Biol.* **11**, 197 (1999).

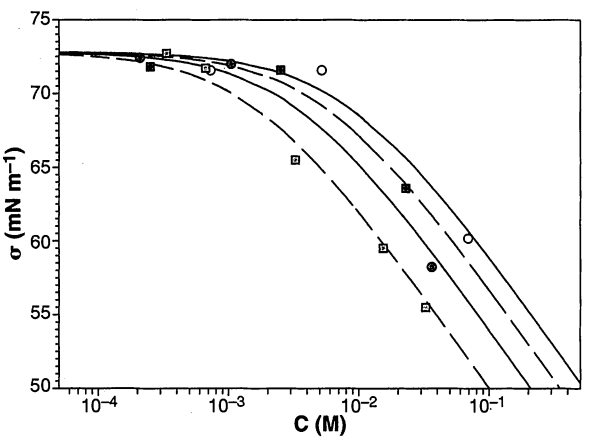
PERSPECTIVES: ATMOSPHERIC SCIENCE

Reshaping the Theory of Cloud Formation

Robert J. Charlson, John H. Seinfeld, Athanasios Nenes, Markku Kulmala, Ari Laaksonen, M. Cristina Facchini

Droplet clouds are the most important factor controlling the albedo (reflectivity) and hence the temperature of our planet. Man-made aerosols have a strong influence on cloud albedo, with a global mean forcing estimated to be of the same order (but opposite in sign) as that of greenhouse gases (*1*), but the uncertainties associated with the aerosol forcing are large. Recent studies indicate that both the forcing and its magnitude may be even larger than anticipated.

Cloud optical properties are controlled by the sizes and numbers of the droplets in the cloud, which are, in turn, governed by the availability of atmospheric particles that serve as cloud condensation nuclei. Twomey (*2*) suggested that an increase in atmospheric aerosols from anthropogenic emissions would lead to smaller cloud droplets because the same amount of cloud liquid water is distributed among more condensation nuclei. For the same liquid water content, a cloud with more numerous, but smaller, drops has a higher albedo than one with fewer, larger drops. This phenomenon, termed the first indirect climatic effect of aerosols, could constitute a major climate forcing (*1*). But current estimates of indirect aerosol radiative forcing or of its uncertainty (*1*) do not include the combined influences of some recently identified chemical factors, each of which leads to additional negative forcing (cooling) on top of that currently estimated.



Surface tension lowering by organics in cloud water. Surface tension decrease with respect to pure water as a result of water-soluble organic carbon in cloud water (expressed as moles per liter of carbon). Data from Tenerife (Spain) and Po Valley (Italy) taken by one of the authors (M.C.F.).

Estimates of the indirect climatic effect of aerosols are based on the theory of cloud droplet formation advanced by the Swedish scientist Hilding Köhler in the 1920s and 1930s (*3*, *4*). Köhler assumed that clouds consist of "activated" water droplets that grow spontaneously after they have reached a critical size corresponding to a critical value of the supersaturation of water vapor. Köhler further assumed that the aerosol is composed of a completely soluble salt and that the particles are in thermodynamic equilibrium until the point of spontaneous growth. Indeed, it is still generally assumed that a cloud forms only in a supersaturated water environment with all the solute coming from the particle. It has recently become clear, however, that soluble gases (*5*, *6*), slightly soluble solutes (*7*), and surface tension depression by organic substances (*8*) also influence the formation of cloud droplets, in a manner unforeseen by Köhler.

Nitric acid (HNO_3) is perhaps the most important highly soluble trace gas in the atmosphere. Ample data establish the prevalence of nitrate as a constituent of cloud and fog water in polluted air (*9*–*11*). In the presence of a water-soluble trace gas such as HNO_3, the critical supersaturation for that droplet is lowered as the gas condenses into a growing droplet. Depending on how it is dispersed over the aerosol population, a minute amount of soluble gas can exert a profound effect on the number of activated droplets. A striking consequence of the presence of a soluble trace gas is that clouds or fogs with micrometer-sized droplets may exist even though the droplets have not undergone traditional activation and even though the ambient relative humidity never exceeds 100% (*5*, *6*). Such "pollution clouds" have a higher droplet number concentration and a broader droplet size distribution than "clean clouds" (*12*).

Highly soluble gases are not the only compounds that can affect aerosol activation. The importance of carbonaceous compounds as components of atmospheric aerosols is well established. A variety of measurements have shown that between 20 and 60% of the carbon mass in fine (diameter <1 μm) atmospheric aerosols consists of

R. J. Charlson is in the Department of Atmospheric Sciences and Department of Chemistry, University of Washington, Seattle, WA 98195, USA. J. H. Seinfeld is in the Department of Chemical Engineering and the Division of Engineering and Applied Science, California Institute of Technology, Pasadena, CA 91125, USA. A. Nenes is in the Department of Chemical Engineering, California Institute of Technology, Pasadena, CA 91125, USA. M. Kulmala is in the Department of Physics, University of Helsinki, 00014 Helsinki, Finland. A. Laaksonen is in the Department of Applied Physics, University of Kuopio, 70211 Kuopio, Finland. M. C. Facchini is at the Instituto di Scienze dell' Atmosfera e dell' Oceano-CNR, via Gobetti 101, 40129 Bologna, Italy. E-mail: seinfeld@caltech.edu