branes that regulate ionic permeability (such as the inner bacterial membrane and endoplasmic reticulum membrane) cannot withstand any measure of ionic leakage. As with pIV, which should only open as the phage are being assembled for export, tightly coupling the opening of ionic permeability channels to substrate transport is the solution to the leakiness problem. The protein-conducting channels of the endoplasmic reticulum and bacterial inner membrane (*8*) are gated open by the signal peptide of the translocating protein (see the figure). In the absence of protein, these channels do not conduct, and there is no danger of ions leaking out. In this way, proton gradients are maintained across the bacterial inner membrane and calcium gradients across the endoplasmic reticulum.

Can the lipid-protein complexes envisioned for fusion pores also mediate the transport of folded proteins across single membranes? During apoptosis, permeation of proteins sequestered in the space between the inner and outer mitochondrial membranes is considered to be the crucial commitment step in the cell death pathway (see the figure). Pro-apoptotic proteins, such as Bax, may promote the formation of pores that are at least partly composed of lipid (*9*). Pore size is dynamically determined by a balance of forces between those that discourage the formation of new edges (linear tension) and those that pull the pore open (for example, mitochondrial swelling). Thus, these putative pores can easily accommodate folded proteins and can even initiate the lysis of a membrane. Alternatively, Bax may open some pIV-like channel in the outer mitochondrial membrane.

The latest mystery of protein translocation involves the HIV transcription factor Tat (and the *Drosophila* protein ANT). Chimeric proteins containing Tat, when misfolded, will move across the plasma membrane directly into the cytoplasm (*10*). Because Tat is highly charged, it is unable to traverse the lipid bilayer of the cell membrane without going through some aqueous or proteinaceous pathway; the dielectric constant of the lipid membrane poses a formidable barrier to the entry of any charged moiety. Perhaps Tat is able to trigger the opening of a hitherto unnoticed megachannel in the eukaryotic plasma membrane. Alternatively, Tat may induce the bending of lipids into transient, localized pores that allow the passage of proteins; the lipids can then seal behind the protein as it passes through.

Regardless of the mechanism of Bax activity and Tat translocation, the Marciano study has now expanded our view of how protein channels in living membranes can allow passage of macromolecular complexes. Now what? We do not know how f1 (and other nonenveloped viruses such as poliovirus) enter cells. Does negatively charged f1 phage DNA induce a pore in the inner bacterial membrane in order to reach pIV in the outer membrane? Are lipidic pores, clearly seen in purely phospholipid membranes, regulated by proteins in biological systems? To paraphrase Hamlet: There are more things in membranes than are dreamt of in our textbooks.

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PERSPECTIVES: HOMOGENEOUS CATALYSIS

Toward Greener Chemistry

R. Thomas Baker and William Tumas

The "greening" of global chemical manufacturing by minimizing energy consumption and waste production has become a major concern for the chemical industry (*1*). New catalyst systems that allow for rapid, selective chemical transformations as well as effect catalyst and product recovery will have a significant impact. Homogeneous catalysts, where the catalyst is in the same phase as the reactants, have some advantages for optimizing catalytic systems, because they can easily be modified by ligand design and their structure and reaction pathways can be characterized in detail by a range of spectroscopic techniques. They are used in a number of commercial applications, but the difficulty of separating the catalyst from the product creates increasing economic and environmental barriers to their broader application.

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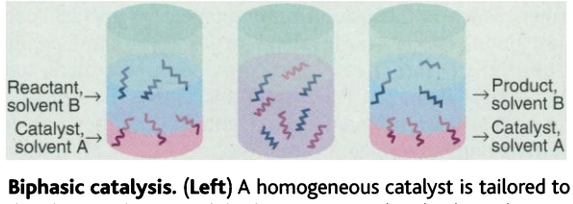
Recently, new strategies have been advanced in which the catalyst is immobilized in a liquid phase that can be separated from the product-containing phase. These novel approaches in so-called biphasic catalysis (*2*) involve designing ligands or engineered soluble supports for catalyst metal centers for high solubility in certain solvents. Solvent systems include fluorocarbons, special mixtures of organic solvents, supercritical CO_2, and ionic liquids. Enhanced aqueous phase systems have also been developed. These fundamental advances in facilitating separation have now sparked renewed interest in developing homogeneous catalysts for efficient synthesis of a wide range of chemicals in the pharmaceutical and chemical industries (*2*).

In an ideal phase-separable or biphasic catalysis system (see the figure on this page), the catalyst and associated ligands would be dissolved in one phase and the reactants and products would be completely soluble in a second phase, which can be removed after reaction and the catalyst phase recycled for further use. To capture the attributes of both a biphasic system and a homogeneous single-phase system, the ideal system would allow for excellent mixing, efficient transfer between phases, or complete miscibility of the phases under the reaction conditions to achieve high reaction rates.

Two approaches have recently been advanced for realizing a totally miscible (one phase) reaction medium under reaction conditions and achieving subsequent efficient separation of phases by cooling the reaction mixture. Both use at least two solvents



Biphasic catalysis. (Left) A homogeneous catalyst is tailored to dissolve in solvent A, while the reactant is dissolved in solvent B. **(Middle)** At the reaction temperature, catalyst, reactant, and solvents A and B form a single phase in which the reaction takes place. **(Right)** After the reaction is completed, the system is cooled down, resulting in phase separation. The catalyst and product are in separate phases, facilitating separation.

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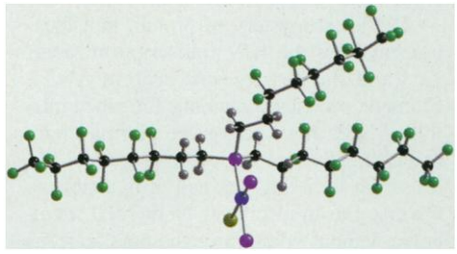
whose miscibility is temperature dependent. Horvath, Gladysz and co-workers have advanced a new concept called "fluorous catalysis" in which fluorocarbons are used to support a homogenous catalyst with highly fluorinated ligands (*3, 4*). Teflon-like "ponytails" are chemically attached to phosphine ligands bound to a metal center (see the figure on this page). The perfluoroalkyl group imparts high solubility in fluorocarbon solvents, and the methylene (CH_2) spacers insulate the phosphorus center from the electron-withdrawing fluoro-substituents. Heating hydrocarbon/fluorocarbon mixed solutions of these catalysts and substrates results in a single phase and effective catalysis for several reactions. After the reaction is complete, the system can be cooled for phase separation. These workers have recently reported an elegant demonstration of "fluorous catalysis, using a rhodium catalyst to effect the addition of a boron-hydrogen bond of catecholborane across the carbon-carbon double bond of an alkene to give an alkylboronate ester which can be oxidized to an alcohol in a second step (*5*). Remarkably, this system does not require a hydrocarbon solvent. High turnovers result in a biphasic system of fluorocarbon solvent and immiscible reactants upon heating. Another recent application of fluorous catalysis takes advantage of the high solubility of dioxygen in perfluorocarbons to conduct catalyzed aldehyde oxidations with the use of nickel complexes with fluorinated acetylacetonate ligands (*6*). The nickel catalyst was recycled up to six times with only a 17% loss of activity.

The second approach, advanced by Bergbreiter and co-workers, uses a thermomorphic solvent system that changes thermally from biphasic to single phase, along with a soluble polymer catalyst support that preferentially dissolves in one phase (*7*). Water-soluble poly(*N*-isopropylacrylamide) was modified to incorporate diphenylphosphino ligand moieties that bind rhodium or palladium catalytic centers. The Rh system, for example, catalyzes the hydrogenation of alkenes with activities rivaling those of traditional homogeneous catalysts. The solvent system consists of equal parts heptane and ethanol with added water, which is biphasic at room temperature but forms a single phase at 70°C. Complete catalyst recycling has been demonstrated for the Rh system and also for a Pd system that catalyzes the coupling of dialkylamines cinnamyl chlorides.

Given the relatively high costs of these solvents and ligands, industrial applications of these two biphasic systems for commodity chemicals will likely require further advances. They may find more immediate use in high-value applications such as pharmaceuticals and fine chemicals. Both are already used in catalyst or reagent immobilization in combinatorial discovery chemistry (*8*).

These approaches, although particularly interesting because of high reaction rates and good catalyst recovery, are by no means the first or the only biphasic catalysis systems. The first commercial example of biphasic



Fluorous catalysis. Model of the fluorous Rh catalyst precursor described in the text. Fluorous tails on two of the phosphorus ligands are removed for clarity. Black, carbon; green, fluorine; gray, hydrogen; blue, rhodium; purple, phosphorus; and yellow, chlorine.

catalysis was the Shell Higher Olefin Process (SHOP) for the production of alpha olefins from ethylene using a nickel-based catalyst in a two-phase butanediol/hydrocarbon reaction medium (*9*). Multiphase concepts with an aqueous phase and water-soluble catalysts were proposed in the early 1970s and spurred significant industrial research (*2*). Aqueous biphasic catalysis is applied commercially on a large scale (600,000 tons per year) in Germany and Korea in the Ruhrchemie/Rhône Poulenc process for the hydroformylation (reaction with carbon monoxide and hydrogen) of propylene to butyraldehyde with water-soluble Rh catalysts (*10*). In the last decade, many other aqueous-phase catalyst systems have been developed for a wide range of transformations of importance to the pharmaceutical and commodity chemical industries (*11*, *12*).

Although aqueous biphasic catalysts are appealing because of the "green" nature of water as a solvent, the contact of organics with process water can lead to the generation of large volumes of dilute aqueous organic waste streams, which are particularly difficult to manage or treat. This increasing environmental concern has led to renewed interest in more environmentally benign solvents, particularly supercritical carbon dioxide and ionic liquids. When compressed and heated above its critical point (73 atm and 31°C), CO_2 forms a highly compressible supercritical fluid with properties intermediate between those of liquids and gases. Catalyst researchers seek to capitalize on the properties of supercritical CO_2-such as pressure-dependent density and solvent properties, lack of toxicity, nonflammability, and miscibility of gases—many of which have been exploited in chromatographic and extraction applications (*13*) (for example, commercial coffee decaffeination). CO_2 and fluorocarbons share similar solvent characteristics as a result of their low cohesive energy densities, and thus there have been parallel efforts in developing catalysts soluble in them. Recent reports show that homogeneous Rh complexes with fluorous ponytail phosphines catalyze the hydroformylation of long chain olefins to linear aldehydes with high selectivity and reasonable rates in fluorocarbons (*14*) and CO_2 (*15*). Recent announcements by DuPont of scale-up activities for fluoropolymer processing initially advanced by DeSimone and co-workers (*16*) demonstrate the potential commercial viability of processing in supercritical CO_2. In our own laboratory (*17*), we are involved in designing CO_2-soluble phosphine ligands with a controlled steric and electronic environment around the ligand, the attachment of ligands to CO_2-soluble polymers (*16*), and the use of water-in-CO_2 micelles (*18*) to increase the rates of phase-separated, water-soluble catalysts with nonpolar organics.

Another approach to biphasic catalysis uses room-temperature ionic liquids, such as alkyl imidazolium salts (*19*). These fluids lack a measurable vapor pressure and can provide useful chemical environments for reactions. Brennecke, Beckman, and coworkers (*20*) recently showed that organics can be extracted from ionic liquids with the use of supercritical CO_2. This result is particularly important, because, as opposed to organic solvents, the nonvolatile ionic liquids are not soluble in the pure CO_2 phase.

Rapid development of catalyst immobilization in solvent systems has led to important and potentially commercial applications. The need for facile separation with near quantitative catalyst recovery will continue to put very stringent requirements on the ligand, catalyst, and solvent system. In addition to catalyst design, a more fundamental understanding of phase behavior, energetics and thermochemistry, partitioning, and the dynamics of catalyst leaching is required. For systems that remain multiphase throughout the reaction, it is important to determine whether the chemical reaction occurs in one of the bulk phases or at the interface, and mechanisms for enhancing mass transport must be defined. Finally, engineering concepts such as reactor design and system modeling will have to be applied.

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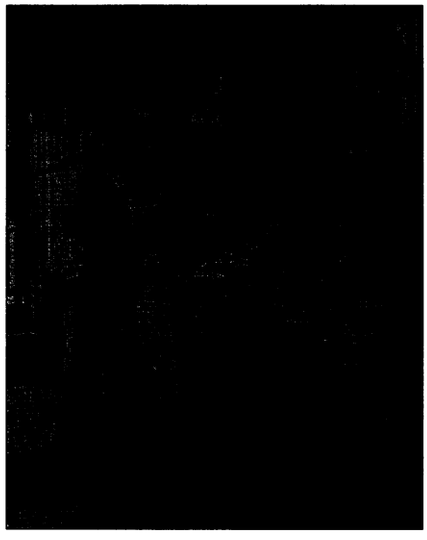
PERSPECTIVES: MICROBIOLOGY

TB Vaccines: Global Solutions for Global Problems

Douglas B. Young and Brian D. Robertson

Tuberculosis remains one of the 10 most important causes of premature mortality worldwide, claiming over 2 million lives every year. In spite of dramatic successes in control of the disease in prosperous countries, geography is an unreliable correlate of protection. Recent sporadic increases in the incidence of tuberculosis (particularly drug-resistant forms of the disease)—most strikingly in New York City—attest to the ability of the causative pathogen, *Mycobacterium tuberculosis*, to exploit new opportunities for travel and immigration and to seek out the disadvantaged pockets in all societies. Control of tuberculosis must be at the global level, with the best prospects offered by improved diagnosis and treatment together with prevention by effective vaccination (*1*). The search for an effective tuberculosis vaccine has challenged and frustrated generations of scientists. In their report on page 1520 of this issue, Behr *et al.* (*2*) exploit state-of-the-art DNA microarray technology to provide new insights into this longstanding problem.

The current tuberculosis vaccine—the Bacille Calmette and Guérin, BCG—was derived from an isolate of *Mycobacterium bovis* (which causes bovine tuberculosis) that had been attenuated by laboratory passage in the early years of this century. It has had a chequered history in efficacy trials, providing more than 70% protection in trials in the United Kingdom, no significant protection in South India, and intermediate levels in a range of other studies in different countries. Behr and Small have previously suggested that differences in efficacy might have arisen as a result of changes in the vaccine strain over time (*3*). Their new study provides further support for this notion, demonstrating that the subculture of BCG in different laboratories resulted in a series of genetic deletions and the evolution of a number of BCG substrains. The authors compared the genomes of *M. bovis* and contemporary BCG strains with that of a virulent reference *M. tuberculosis* strain, using comparative hybridization to a DNA microarray. They found that Calmette and Guérin "lost" a 10-kilobase fragment from the *M. bovis* progenitor strain during the initial process of attenuation. A second fragment was deleted in the late 1920s, and three additional fragments were selectively deleted during subsequent passage in separate laboratories to generate the current diaspora of BCG substrains. Although there is as yet no direct evidence to confirm Behr and Small's hypothesis that these deletions are responsible for changes in vaccine efficacy, the results provide a rational starting point for attempts to generate—or perhaps regenerate—a better BCG vaccine.



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A tuberculosis patient in a Victorian hospital.

These observations represent an important addition to knowledge of BCG deletions originally identified in previous groundbreaking publications (*4*, *5*). A key aspect of the present study is that it provides the first such analysis at a whole-genome level. The authors have used information from the recently completed genome sequence of *M. tuberculosis* (*6*) to construct a DNA microarray in which almost every open reading frame is displayed. This has allowed a global analysis of genetic differences between *M. tuberculosis*, *M. bovis*, and the various BCG substrains. Almost 100 *M. tuberculosis* genes were "missing" from all of the *M. bovis* isolates that were examined. Earlier sequence-based analysis of a limited set of genes demonstrated a remarkably high degree of genetic conservation between *M. bovis* and *M. tuberculosis* (*7*). The new findings suggest that genetic diversity amongst members of the *M. tuberculosis* complex (comprising *M. tuberculosis*, *M. bovis*, *M. africanum*, and *M. microti*) may in fact be much greater than previously anticipated, and that gene deletion, rather than point mutation, may be a key source of this variation. Currently, information provided by the microarray approach is "one-way," identifying *M. bovis* deletions relative to the framework provided by the *M. tuberculosis* genome. Sequence analysis of the genome of *M. bovis* is currently under way (*8*), and identification of regions present in *M. bovis* but absent from *M. tuberculosis* will also be of considerable interest in the context of understanding the evolution of the bovine and human pathogens. As in the case of the BCG vaccines, functional analysis of the "missing" genes may provide important insights into mycobacterial physiology.

In addition to the genotypic analysis described by Behr *et al.*, the microarray approach is also applicable to the study of temporal changes in global patterns of gene expression. This is accomplished with the use of RNA isolated from organisms grown under different conditions and has been successfully applied to the investigation of global changes in gene expression associated with the shift from fermentation to respiration in yeast (*9*). This approach has also yielded information about gene expression

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