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PHARMACEUTICAL SCIENCES IN THE NEXT MILLENIUM

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INTRODUCTION

Paul Ehrlich wrote in 1913:

"Now that the liability to, and danger of, disease are to a large extent circumscribed – the effects of chemotherapeutics are directed as far as possible to fill up the gaps left in this ring".

Optimistic words indeed. Ehrlich was, of course, quite unable to predict the new viruses, bacterial resistance to antibiotics, the spread of tropical diseases and the consequences of unchecked population growth. These are our four contemporary horsemen of the Apocalypse:

"And I looked, and behold a pale horse. And his name that sat upon him was death"

Revelations 6:8

These and other diseases and potential new epidemics will challenge the pharmaceutical sciences in the next decade as, perhaps, never before.

There is, however, clear evidence that the current and future importance of the pharmaceutical sciences is realized. Frank Press observed in 1992 that:

"Twelve technologies are predicted to be critical to our forthcoming competitiveness. Included in the Life Sciences are "bio-processing", "drug design" and targeted pharmaceuticals".

The Scientist, 1992.

Subsequent events suggest that these were indeed prescient words that bode well for the continued rapid and necessary growth of the pharmaceutical sciences. Moreover, there is continued encouragement in the rapid development of drug discovery through advances in combinatorial chemistry, screening methodologies and genomics – all linked through bio-informatics. And it is increasingly clear that functional genomics will play a determinant role in the shaping of the direction of both pharmaceutical science and its therapeutic application.

It is also clear, however, that the direction of the pharmaceutical sciences will be shaped not only by science, but will also be significantly impacted by economic, ethical and political and public issues [Table 1]. Indeed, these issues may ultimately be more challenging than even the necessary scientific advances. The impact of the economics of health care costs and delivery in both developed and developing worlds will be a major driving force towards the reduction of the costs of therapeutic agents and services. The present cost of developing a molecule to the clinic is estimated to be in excess of $350

million and rapidly increasing: this is a non-sustainable trend. Additionally, the time of exclusivity between the introduction of a new molecule and the advent of competition is rapidly decreasing and the windows of technology are closing ever more rapidly. However, increased population growth and demographically-driven driven demands from the burgeoning very young and very old will be factors that will simultaneously escalate demand. Global competition and the potential migration of established diseases as a consequence of habitat destruction and global warming will constitute another set of major and world-wide social challenges. Ethical considerations arising from the availability of techniques from gene replacement, germ line modification and cloning will demand resolution at this time, and this, together with the increased availability of individual genetic information and the ability to read this information for inherited and trait characteristics will further increase ethical stress and public concerns.

TABLE I.

FACTORS IMPACTING THE FUTURE OF PHARMACEUTIAL SCIENCE

- Science
- Science support
- Education policy
- National and global economic policies
  - Competition across the borders
- Healthcare policies
  - Economics
  - Politics
  - Public demand
  - Ethical issues
- Environmental issues
  - Habitat destruction
  - Global warming

All of these factors are important and all will mutually interact. This presentation will, however, focus on only one factor, the impact of scientific development, and specifically the relatedness of two fundamental components of pharmaceutical discovery - synthetic chemistry and molecular biology. Increasingly, the fundamental paradigms of molecular biology and chemistry seen as random, amplification-driven and diverse *versus* selective, yield-based and specific respectively are becoming complementary and mutually reinforcing.

THE IMPACT OF MOLECULAR BIOLOGY

The paradigms of molecular biology, as a mature and integrated discipline, will be pervasive in the next millenium: the gen(I)e is out of the bottle, and the issue is not simply whether there will be a 21st Century Therapy Mall where one will shop for Designer Jeans and Designer Genes, but rather the impact of genomics on all facets of the drug discovery and development process [1]. From gene therapy and replacement to

pharmacogenetics – the correlation of individual variation in responses to drugs caused by genetic polymorphisms – genomics will transform 21st Century therapeutics. These techniques will revolutionize not only patient treatment, but also the conduct of clinical trials and the selection of patients for specific therapies [2]. We have made the following transition:

- Gene product as drug
- Gene product as tool for drug design
- Gene itself as drug
- Gene and drug therapy *integrated*

The closing of the Glaxo-Wellcome's Geneva Research Institute this year on the grounds that "...no longer need a "stand-alone" molecular biology institute" is one indication of how molecular biology is regarded as an integrated discipline.

The existing sequence of drug discovery is depicted in Figure 1. This process, essentially a "one molecule at a time" hand-assembled drug production line, is already in the midst of major change. The cost of bringing one molecule into first clinical use is currently estimated at approximately $350 million. This cost is likely to increase significantly as therapeutic targets, including neurodegenerative diseases, become increasingly expensive both in terms of discovery science *and* clinical evaluation unless new approaches are employed. These approaches include the new enabling technologies of combinatorial chemistry and high throughput screening and functional genomics These technologies are linked together by the "glue" of bio-informatics [Figure 2].

In particular, the influence of molecular biology is being seen very dramatically at the beginning of this process - chemical synthesis and the search for lead molecules. Continued influence may lead to a new paradigm of chemistry with molecules that are self-synthesizing, self-evolving, self-targeting and self-limiting, and which through the association with nanotechnology become the 21st Century version of Ehrlich's magic bullet.

Molecular Discovery.

"The achievements of chemical synthesis are firmly bound in our attempts to break the shackles of disease and poverty"

Roald Hoffmann, 1993

Listening to nature. The medicinal chemist is constantly on the search for new structures that will serve as leads for new drugs. Increasingly this search leads to natural products. These molecules are often biologically active in their own right and are

structures created in the crucible of evolutionary and selectional pressures. They are frequently molecules of substantial structural novelty that both challenge the creativity of the chemist and stimulate new pathways of synthetic chemistry. Some recent examples of novel natural structures include the "taxol mimics" eleutherobin and sarcodictyin A [3,4]. The recent synthesis of brevetoxin A, a potent neurotoxin secreted by *Gymnodinium breve* and associated with "red tides", provides a further contemporary example of the importance of synthetic chemistry : a highly complex molecule with 22 asymmetric centers its synthesis provides further intellectual stimulus for the necessary synthetic creativity that will increasingly underlie drug discovery [5].

In recent years the public, science and government and regulatory agencies have become increasingly sensitive to issues around alternative or complementary medicine, including herbal medicine. As a consequence there is renewed interest in plant and animal sources for new molecules [6].

The Amazonian tree frogs of the genus *Dendrobatidea* have yielded many novel and pharmacologically active alkaloids [7]. From the Ecuadorian frog *Epipedobates tricolor* has been isolated epibatidine a potent and receptor subtype-selective agonist at nicotinic receptors. Epibatidine has served as a structural lead for ABT-594 [R-5-(2-azetidinylmethoxy)-2-chloropyridine, a potent non-opiate analgesic that acts selectively at neuronal nicotinic receptors to mediate this analgesic effect [8].

The importance of such discoveries places new emphasis on biodiversity. Although we have but a poor understanding of the number of species – there are an *estimated* three to five hundred million species, and that of these only 0.00002-0.003% are used as herbal medicines [9]. Thus, there is, in principle, a major opportunity for drug discovery among the non-explored species. However, there is simultaneously an increased concern over the rapid loss of biodiversity and potentially valuable species. Species loss is, however, not a uniquely 20th Century problem. We have likely lost valuable plant-based medicines in previous era. One example is the plant called *silphion* by the Greeks that grew specifically in the North African city-state of Cyrene and was recognized as an effective female contraceptive agent. Over-harvesting led to its apparently complete extinction [10].

Chemical diversity. Combinatorial chemistry in its various guises has clearly been an enormously valuable technique, when combined with high throughput screening, has led both to new leads and to the molecular exploration of lead "space". This technique mimics the ability of Nature to generate molecular diversity and, in the case of phage expression techniques, allows endogenous biosynthetic machinery to generate biodiversity [11]. In principle, there are few limits to the number of molecules that may be made combinatorial methods: in practice, there are significant limitations not least of which is the necessity for powerful informatic systems with which to keep track of data in easily retrievable and manipulatable form and the necessary limitations on the numbers of molecules that can be examined for biological activity.

These considerations suggest that combinatorial chemistry can mimic nature more closely by linking more directly the generation of molecular diversity with *both* biological selection [screening] *and* molecular evolution linked to biological fitness. The cone snails [*Conus* genus] provide an interesting potential example of this strategy. These snails generate disulfide-bridged toxins of rigid three-dimensional structure that are potent at and selective for a number of membrane receptors and ion channels: several of these agents have become valuable pharmacological tools and potential therapeutic agents. Conus appears to follow a combinatorial biochemical approach in which the active peptides are produced as larger precursors with a stable N-terminus and a hypervariable C-terminus [Figure 3]. With the evolution of new *Conus* species the endogenous peptide sequences have progressively diverged and it may be speculated that the active sequences now present represent the "fittest" molecules with the inactive deleted by natural selection [12].

The provision of unnatural amino acids to "expand" the genetic code offers further potential for increasing the chemical repertoire [13]. This process takes advantage of the relatively broad substrate specificity of the translational machinery and by replacing a codon for a particular amino acid with a specific stop codon and supplying a suppressor tRNA acylated with the "unnatural" amino acid that recognizes this codon a general biosynthetic method for synthesizing novel peptides is available.

The exploitation of combinatorial biosynthesis has recently been documented for the polyketide family, synthesized by bacteria and fungi and which includes numerous antibiotics, antifungals and anticancer agents. Amongst them are the following important biopharmaceuticals – avermectin, erythromycin, oxytetracycline and candidicin. These agents are difficult to synthesize, particularly on the large scale that would be needed for pharmaceutical production. Engineering of the modular polyketide synthase permits the introduction of alternative substrates and the generation of structurally novel macrolides [Figure 4]. Additionally, the choice of appropriate alternative substrate yields macrolides that are suitable for conventional chemical modification [14,15].

Self-reproducing molecules. Self-replicating structures or "molecular machines" are an increasingly studied area of research. In addition to the obvious implications for the origin of life during a pre-biotic phase of evolutionary history, there are important implications for the realization of molecular-level manufacturing devices in the field known as nanotechnology.

Increasingly complex self-reproducing molecules are being described. A number of systems of small molecule reproduction have been described based on the self-complementarity of nucleic acid-like base pairing interactions. In such systems assembly of substrate via complementary hydrogen-bonding on the template molecule demonstrates sigmoidal growth [Figure 5]. These systems can also show behavior that incorporates "mutation" and "evolution" into generating more efficient replicators [16,17]. For example, the template might be light sensitive and under the influence of light might form a better template for the substrates A and B and would then "compete"

more effectively for substrate. More recently, self-reproducing peptides have been described that extend this paradigm beyond these base-pairing systems. Thus, a 32-residue *alpha*-helical peptide based on the leucine-zipper domain of a yeast transcription factor serves as the template and auto-catalyst for promoting the condensation of 15- and 17-residue fragments in neutral, dilute aqueous systems [18].

Evolving molecules. Biologically-driven chemical synthesis is subject to constant evolutionary pressures: as a consequence the diversity of chemical production is harnessed to biological fitness. The translation of this cellular paradigm to the *in vitro* or "test tube" environment – *"directed evolution"* - is being realized in a number of systems [19]. Mutation of a molecule to produce a large number of variants is coupled to a selection process that defines a specific binding or biological activity: molecules that satisfy these criteria are then subject to further rounds of mutation and the "fittest" molecule is ultimately selected. Thus, random variation of an eight base sequence of an RNA that interacts with a specific protein yields a theoretical total of 65,536 individual species from which were selected two ligands of equal affinity – the wild type and a novel sequence differing from the wild-type at 4 positions [20] Similarly, an RNA-cleaving enzyme [ribozyme] was converted to a DNA-cleaving enzyme in ten rounds of amplification, selection and mutation [21].

Self-targeting molecules. Since Ehrlich's time the goal of the pharmaceutical sciences has been the "magic bullet" targeted only to those specific cells or pathways that are defective. This can be approached by specificity of molecular design and by taking advantage of biochemical pathways and processes that may be uniquely present or absent in the disease state. This specificity of interaction can be enhanced or even created by engineering the targeting pathways.

The introduction of a retroviral vector expressing herpes simplex thymidine kinase into experimental gliomas rendered them sensitive to the anti-herpes drug ganciclovir. This process exploits the property of retroviral vectors to transfer genes only into dividing cells and thus make them uniquely sensitive to a specific drug [22]. More recently, the necessity of HIV to use two co-receptors – CD4 and a chemokine receptor – to enter and to infect immune cells and to assume control of the cells biosynthetic machinery has been exploited to devise a novel anti-viral chemotherapeutic strategy. After HIV infection cells express on their surface viral proteins that are the substrates for the co-receptors and this can be exploited by engineering into a Rhabdovirus these proteins in place of the normal viral envelope protein. These engineered viruses can then fuse only with HIV-infected cells to produce a highly targeted "viraceutical" [23]. With an engineered vesicular stomatitis virus expressing the co-receptors CD4 and CXCR4 and the normal complement of structural core proteins of the virus a viraceutical was produced that not only destroyed cells expressing HIV-1 envelope proteins, but that also propagated to kill of any cells that chose to express this protein – a viral equivalent of Clint Eastwood's "Dirty Harry"! [24].

NANOTECHNOLOGY AND DRUG FACTORIES

Pharmaceuticals that are self-synthesizing, reproducing, evolving and targeting may be suitable for manufacture in nanomachines – molecular machines or bioreactors designed to synthesize specific groups of molecules. Nanostructures are being viewed increasingly as candidate structures for synthesizing and tailoring molecules and may be envisioned as reactors for pharmaceuticals production and operating at the cellular level to synthesize drug molecules "on site" [25,26].

SCIENCE AND PUBLIC POLICY

The scientific policy in the United States post-world war II has been principally that outlined by Vannevar Bush in, *"Science, the Endless Frontier"*. With its essentially linear model linking basic science to technological exploitation it has generated a large and significantly university-driven Federal role for the support of basic research with commercial product technology being funded by industry. In an increasingly competitive environment this model is being challenged. It is likely that the division between "basic" and "applied" research has never been that clear and that there is a large area where the two are very intimately linked [27]. This has long been true for the biomedical and pharmaceutical sciences and part of the reason for the continued and increased Federal support of the NIH is not simply that politicians also get sick, but that the link between basic research and the cure of disease is seen very clearly. This has not been the case for other areas of science, notably the physical sciences and engineering and this has hampered the development of national policies and priorities *and* it has constrained university research in artificial ways. . Indeed, Donald Stokes has recently argued in his posthumously published book, "Pasteur's Quadrant" that the linear model is not only untrue, but that it has significantly constrained national science policy [27]. The pathways linking science and technology are not clear, uncluttered and unidirectional, but rather are "multiple, complex and unequally paced". There are many occasions where science owes more to the steam engine, than the steam engine owes to science.

However, it is likely that changes in the organization and support of biomedical and pharmaceutical research will continue to occur even though the existing model has been very successful. The existing relationships between major pharmaceutical companies, biotechnology organizations and universities will continue to evolve in the direction of more equal and cooperative partnerships. The division between basic and applied research here will become increasingly seamless and develop in the direction of shared university and industry programs and personnel in the direction of the "pharmaceutical research cell" where:

- "Cell entrepreneurs" work out business plan
- "Cell entrepreneurs" obtain funding and obtain financial stake
- "Cell entrepreneurs" lease research facility from university
- "Cell entrepreneurs" sell discovery and move on

After M. Edwards [28].

CONCLUSIONS

Even the cursory survey of this article suggests that the pharmaceutical sciences are being rapidly transformed under the influence of both the new technologies and sciences and the economic imperatives. Of particular importance are scientific and technological advances that may greatly accelerate the critical process of discovery. The possibility of a drug discovery process built around the principles of directed diversity, self-reproduction, evolution and self-targeting suggests a new paradigm of lead discovery, one based quite directly on the paradigms of molecular biology. Coupled with the principles of nanotechnology we may contemplate miniature molecular machines containing directed drug factories, circulating the body and capable of self-targeting against defective cells and pathways – the ultimate "drug delivery machine".

However, science and technology are not the only factors that will transform the pharmaceutical sciences in the next century. The necessary reductions in the costs of drug discovery brought about by the rapidly increasing costs of the current drug discovery paradigms means that efforts to decrease the discovery phase and to make drug development part of drug discovery will become increasingly important. This is likely to involve increasing numbers of "alliances", as well as the creation of *"pharmaceutial research cells"* –highly mobile and entrepreneurial groups within or without a pharmaceutical company that are formed to carry out specific discovery processes. Some of these will be in the biotechnology industry, but an increasing number will be in universities [28]. The linear process from basic science to applied technology that has been the Western model since Vannevar Bush's "Science: The Endless Frontier" has probably never been particularly linear and, in any event, is likely to be rapidly supplanted by models where science, scientific development and technology are more intimately linked [27]. The pharmaceutical sciences have always been an example of use-directed basic research, but the relationships between the pharmaceutical industry, small and large, and the universities seems likely to become increasingly developed in the next century. This may serve as a significant catalyst for the continued transformation of universities into the "knowledge factories" of the 21st Century [29]. Regardless, we may expect to see major changes in the research organizational structure in the

pharmaceutical sciences even as pharmaceutical companies enjoy record prosperity. And this is in anticipation of tough times to come [30].

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FIGURE LEGENDS

Figure 1. A flow sheet for the introduction of a new pharmaceutical agent.

Figure 2. The "new paradigm" of drug discovery.

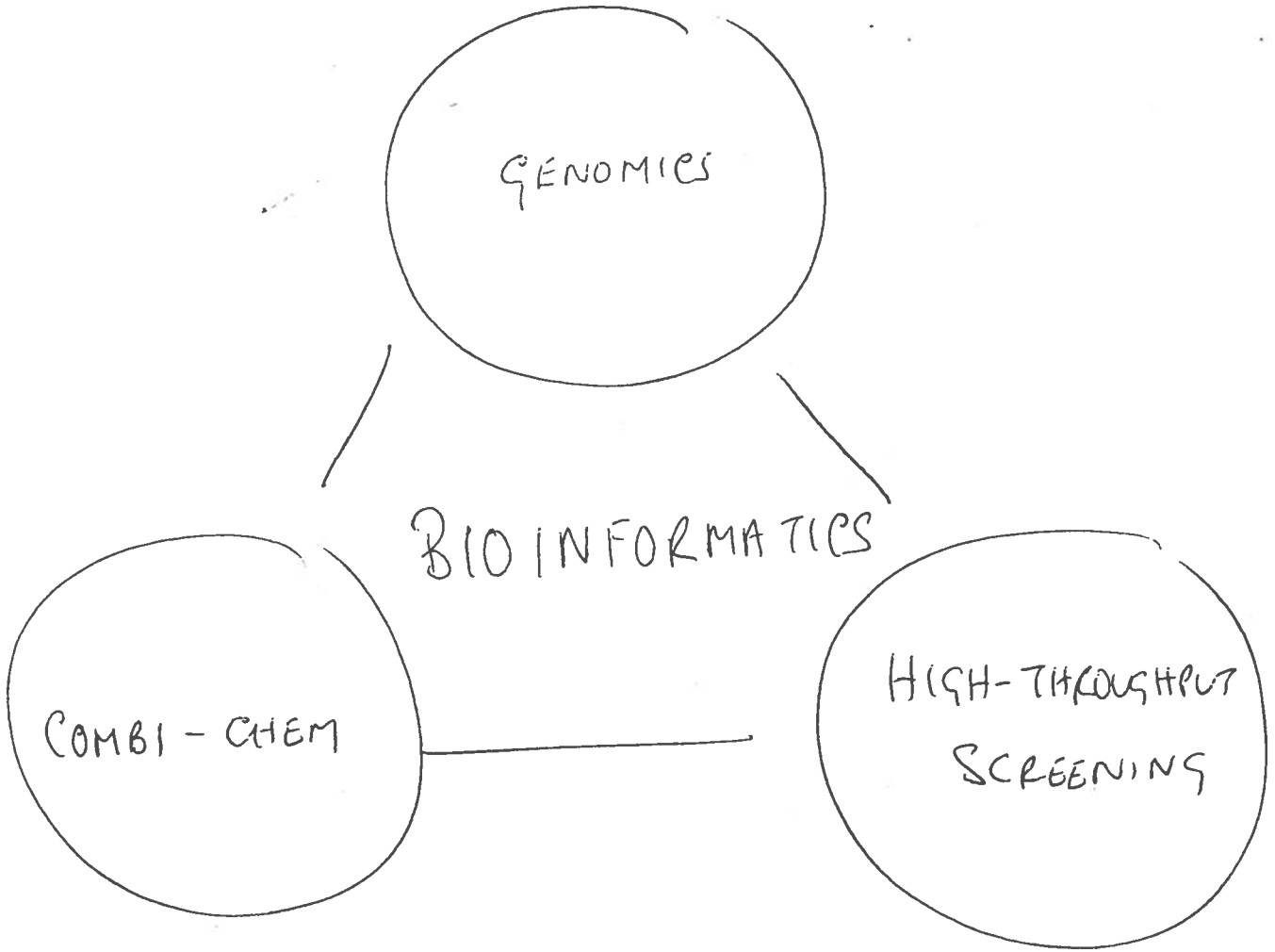
Figure 3. The process of "peptide diversification" during *Conus* speciation. The N-terminal region is constant, but the C-terminal region is hypervariable and generates new peptides that are "selected for" by evolutionary pressure. Reproduced with permission from Olivera *et al.* Trends Biotech., 1995 [12].

Figure 4. The modular biosynthesis of polyketides. The interruption of one enzyme in the assembly line permits the introduction of novel starting materials and the generation of new molecules. Reproduced with permission from R.A. Service, *Science* 277: 319, 1997.

Figure 5. Schematic representation of self-reproducing molecules whereby the formation of template T from precursors A and B generates more template. This process may evolve if, for example, T is light sensitive and is affected by light to create a new template T* that is more effective than T.

New Drug Development

Stage		Time
I	~5,000 molecules Research plan Chemical synthesis/natural product Assays	2-5 years
II	~20 molecules Extensive pharmacology Mutagenicity Structural optimization Back up compounds Optimal agent selection	2-4 years
III	~3 molecules Efficacy Pharmacokinetics Metabolism Toxicology Clinical trials	2-6 years
IV	~1 molecule Marketing Post marketing surveillance	~3 years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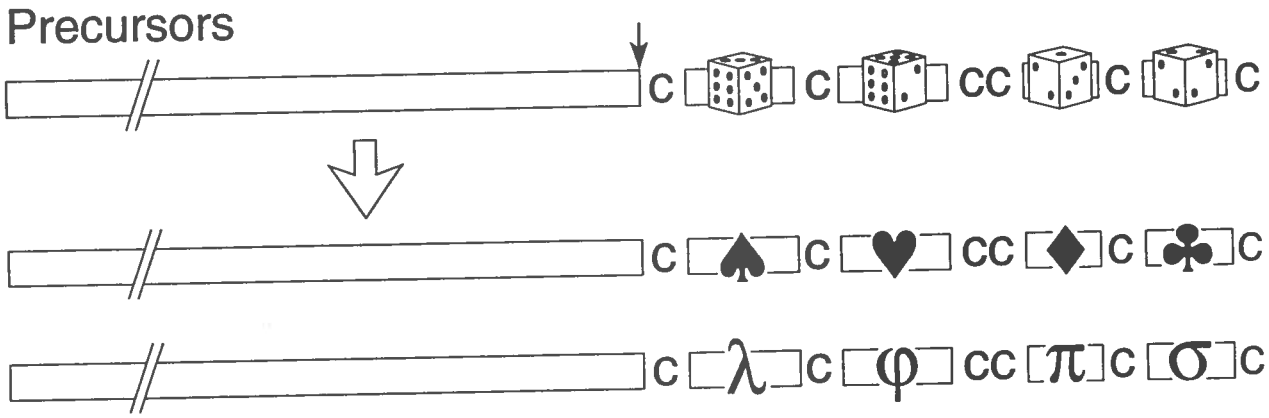
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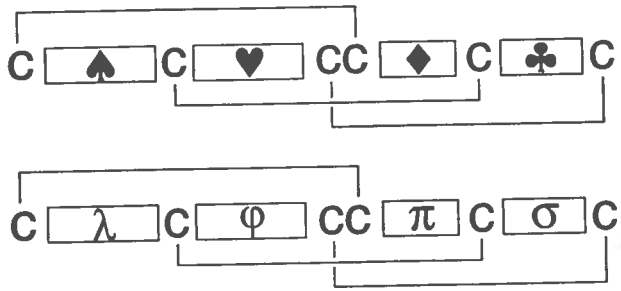
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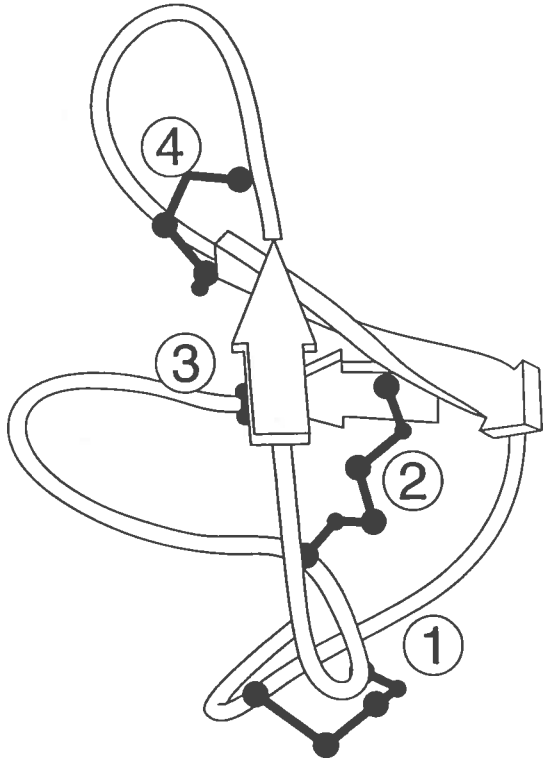
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BIOCHEMISTRY

Hijacking a Cell's Chemical Paths to Make New Antibiotics

The push is on to find new versions of old wonder drugs, as ever more strains of bacteria develop resistance to conventional antibiotics. The trouble is, many antibiotics have a complex molecular architecture that makes them extremely difficult to synthesize, let alone tinker with afterward. For these molecules, medical researchers have largely had to accept what nature gives them: compounds made by antibiotic-producing bacteria and fungi. In this issue of *Science*, however, a group of biochemists describes a way to hijack the antibiotic-producing chemical pathways of bacteria, exploiting them to produce a wide variety of new compounds.

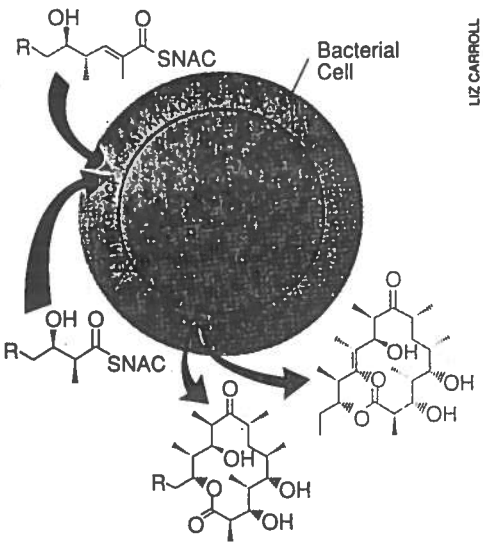
To construct their natural products, these bacteria rely on an assembly line of about 30 enzymes in which each enzyme hands off its produ... Khosla, a chem... iversity in Palo... colleagues have ... embly line partw... ural inter- media... one, and restar... t on page 367, t... up by the enzyn... e growing chem... nging in- terme... t the bugs to co... c analogs, many... g manipu- lated... hal chem- istry techniques.

"It's a nice piece of work," says Leonard Katz, an antibiotics researcher at Abbott Laboratories in North Chicago, Illinois. "It opens the door to making a bunch of new molecules quickly"—molecules that can then be evaluated as potential new antibiotics, antifungals, or anticancer compounds. Katz is doubtful, however, that the technique could be scaled up for industrial production, as it requires researchers to add synthetic starting molecules that may be time-consuming and expensive to make.

Still, just finding candidate compounds is half the challenge in discovering new drugs. To date, one of the richest sources of promising molecules has proven to be a family of complex natural compounds called polyketides, most of which are made by bacteria and fungi with their involved, assembly-line process for use as defensive chemical weapons. Of the thousands of polyketides discovered thus far, hundreds have already been tapped as pharmaceuticals. That success rate has sent researchers looking for ways to generate new polyketide variants.

By tinkering with the genes for the enzymes that are the "workers" in the assembly-line process, scientists can alter the end product. But "it's still a relatively cumbersome process to make modifications by altering an organism's genes," says Khosla. What's more, the variety of novel polyketides this strategy can produce is limited, because the organisms must assemble their compounds from molecular building blocks at hand in the cell.

Chemists, of course, can produce a far richer array of building blocks. So Khosla and his colleagues—Stanford postdoc John Jacobsen, Richard Hutchinson of the University of Wisconsin, Madison, and David Cane of



LIZ CARROLL

Break and switch. By interrupting an enzymatic assembly line and substituting new molecules for the natural intermediate, biochemists can alter the final product.

Brown University in Providence, Rhode Island—decided to give polyketide-producing bacteria some new building blocks to work with. The researchers started with *Streptomyces coelicolor*, an organism that is easy to manipulate genetically. They had previously engineered the bacterium to express the entire series of 28 enzymes needed to make the common polyketide antibiotic erythromycin.

Using conventional genetic-engineering techniques, they disabled the third enzyme in the series. That prevents it and enzymes 4 through 6 from working together to take up pairs of a natural, molecular building block—a small, three-carbon chain compound called propionic acid—and from stitching them together into a single six-carbon chain. Enzyme 7 normally takes up this chain and passes it on down the assembly process. But without this six-carbon chain, "the other enzymes don't get what they need to do their thing, and the whole system comes to a grinding halt," says Khosla.

To reboot the system, the researchers synthesized molecules slightly different from the six-carbon chain that enzyme 7 normally uses as its feedstock and then substituted them for the original. When eight-carbon building blocks were added to the *S. coelicolor*'s fermentation bath, for example, the stand-ins were taken up by enzyme 7 and passed along until a final, new polyketide, possessing two extra carbons, emerged at the end of the assembly line. The result shows that "these enzymes seem to be very tolerant to using new substrates, and that's good news for making novel natural products," says Khosla.

Enzyme number 7 and its downstream brethren even accepted more radical changes, such as one building block with a six-carbon ring linked to a six-carbon chain, transforming these into a variety of new polyketides. A final processing step at the end to add sugar groups that are normally present on erythromycin turned these products into active antibiotics, says Cane. What's more, the activity of these compounds can be fine-tuned, because they have chemical structures not found in natural polyketides, such as double bonds between adjacent carbon atoms. Medicinal chemists are adept at modifying such structures.

Despite this promise, "the technique still has a long way to go before it's ready to produce molecules for market," says Katz. He points out that even though *S. coelicolor* turns out the new products as efficiently as it normally produces erythromycin, other organisms used in commercial manufacture of the drug have been engineered to produce quantities 1000 times larger. And unless the novel polyketides can be produced at this level, it's doubtful the process would be commercially viable, says Katz. He also points out that while erythromycin building blocks are all produced for free as natural metabolites in bacterial cells, the building blocks for the analogs must be specially synthesized, which can be both time-consuming and expensive.

Cane, however, sees no reason why the biochemistry of commercial organisms couldn't be hijacked as well, turning them into factories of new compounds. The need for custom-made building blocks isn't a major obstacle either, Khosla says. He notes that the building blocks he and his colleagues tested took only three or four steps to make, which he calls "within the range of what synthetic chemists are comfortable producing." A few extra steps could be a small price to pay if the strategy leads to new weapons against antibiotic-resistant bacteria.

–Robert F. Service

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$$A + B \longrightarrow T$$

$$T + A + B \longrightarrow 2\,T$$

$$T \xrightarrow{h\nu} T^*$$

$$T^* + A + B \longrightarrow T^* + T$$