How to Find New Antibiotics

| VISION | *Metagenomics could be the way to mine the soil beneath our feet*

By Jo Handelsman

We need new antibiotics. It's as simple as that. We can reduce the need for them by deploying sophisticated surveillance systems and implementing public health policies that reduce the spread of infectious disease. We can prolong the active life of existing antibacterial agents by enforcing their prudent use and devising clever ways to manage resistance. But we will still need new drugs.

Bacteria are responsible for 25% of deaths worldwide; one-third of the world's population has tuberculosis; and in the United States, 98,000 cases of septicemia are reported each year with a 31% mortality rate, thereby killing as many people annually as acute myocardial infarctions. Hospitals have become treacherous breeding grounds for opportunistic pathogens, inducing two million hospital- acquired infections per year.[1] As our world becomes more populous and transportation continues to blur intercontinental boundaries, making the words "indigenous" and "endemic" anachronistic, the rate of spread of infectious disease will accelerate.

Yet there is no significant drug pipeline to help us keep pace. Antibiotics' limited profitability and a misplaced belief that we have conquered infectious disease drove an exodus of pharmaceutical companies from antibiotics research over the past two decades.[2] Wyeth's tigecycline, released this year, is the first new class of antibiotics to hit the pharmacy in 20 years.

Most of the antibiotics used today – erythromycin and vancomycin among them – come from cultured soil bacteria. But, these organisms represent only the tip (0.1%) of a microbiological iceberg; the vast majority of soil denizens are unculturable by standard methods. Given the many antibiotics cultured soil bacteria have already provided us, the rest of the population is certainly worth exploring. Yet, until recently, the technology did not exist for us to do so.

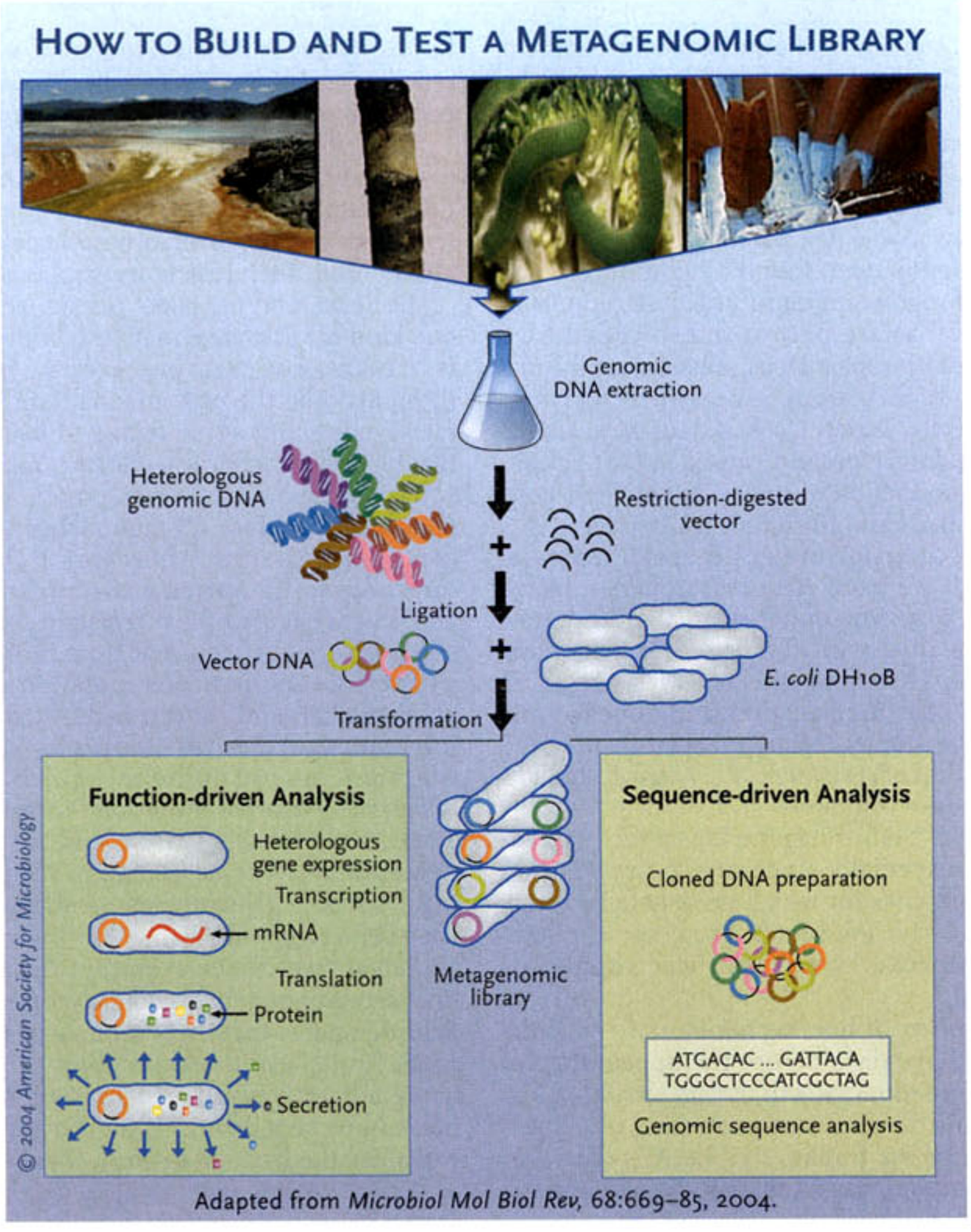
Metagenomics, which is the culture-independent genomic analysis of assemblages of uncultured microorganisms, promises to fill that technological void. Proof-of-principle experiments have already demonstrated the ability of metagenomics to identify novel gene products, including antibiotics. We'll need more federal and private resources if we are to determine the extent of, and ultimately mine, this pharmaceutical vein.

METAGENOMICS DISCOVERY Metagenomics technology involves extracting DNA directly from an environmental sample (seawater, soil, or insect guts, for instance), cutting it with restriction enzymes, and cloning it into a culturable host, such as *Escherichia coli*.[3,4] One of three approaches is typically taken to identify clones of interest.

Some studies have used random sequencing to generate massive databases of DNA sequence. Others have screened clones to find genes of a particular family or that share a particular motif. But with both of these approaches, genes of interest can be identified only if they share sequence similarity with genes that have already been discovered in cultivatable organisms. Novel genes and gene families, by definition, are overlooked by such approaches.

We therefore must also adopt a functional approach. Functional metagenomics involves screening metagenomic libraries for the expression of a function, such as the appearance of a pigment, enzymatic activity, or antibiotic. Because clones in these screens are selected by phenotype (such as expression of an antibiotic activity) and not by homology to some known sequence, the strategy can identify novel genes as well as novel activities of known gene families.

My lab and collaborators used functional metagenomics to discover turbomycin A and B, new antibiotics produced by *E. coli* carrying a fragment of DNA extracted from Wisconsin soil.[5] Julian



Davies' group at the University of British Columbia discovered another new antibiotic, terragine, by expressing environmental DNA in *Streptomyces lividans*.[6] Neither of these activities would have been recognized by sequence analysis alone, and importantly, we don't know the bacterial source of either compound.

But turbomycin and terragine are mere tracer gold. If there's a richer vein of antibiotics beneath our feet, we'll need bigger, more expansive studies to find them. Such studies won't be easy, however.

DAUNTING TECHNICAL HURDLES Though genes from a surprising diversity of organisms have been expressed in *E. coli*, many more functions await discovery in these libraries. That's because genes that originate in organisms that are phylogenetically distant from the host organism used for cloning and screening may not be expressed. This barrier can be addressed, in part, by screening libraries in multiple host species with different transcriptional regulators, codon usage, or other features that affect gene expression.

Another limitation is that all of the auxiliary functions necessary to synthesize the antibiotic must reside in the host cell. Most often, antibiotic biosynthesis genes are clustered on contiguous genomic fragments. But, genes for the production of specialized cofactors or substrates might not be linked to the biosynthetic pathway, thus making it impossible to capture all of the necessary machinery for synthesis in a single clone.

The most common method for screening metagenomic libraries for antibiotics is to replica-plate the clones onto a bacterial lawn and look for "zones of inhibition" (i.e., regions devoid of bacterial growth). Such assays are relatively insensitive, however, because sufficient antibiotic must be produced and secreted by the clone to inhibit bacterial growth, and they are too laborious for screening large libraries. Yet they prove the richness of the source: If a few proof-of-concept studies have already yielded new antibiotic candidates, imagine what a concerted, large-scale effort might produce.

Tapping that capacity, however, is daunting. Microbial communities may contain thousands of species and many more strains. It is likely that more than 200 gigabases of cloned DNA is needed to obtain eightfold coverage of the soil metagenome. With inserts averaging 10 kb, such a library would contain 20 million clones. If these are then transferred to multiple host species to maximize gene expression, it might be necessary to screen one billion clones. Classical screens cannot handle even a fraction of such a library.

Academic researchers have made some progress in developing high-throughput screening strategies. In January, Taku Uchiyama of the Marine Biotechnology Institute in Iwate, Japan, published an intracellular screen in which a biosensor for the desired activity resided in the same cell as the metagenomic DNA; the clones were then sorted rapidly to identify those of interest.[7]

The pharmaceutical industry could augment these efforts and accelerate the pace of metagenomics. It has both the ingenuity and the technical capacity to develop and implement screens at this level—after all, it was their brute-force efforts in the 1940s and 1950s that gave us many of the antibiotics we use today. But they need incentives to do so: antibiotics do not provide the financial enticements of drugs used for such chronic conditions as asthma, high blood pressure, and diabetes.

A FAIR TEST It will take more than industrial ingenuity to strike metagenomic gold. The complexity of microbial communities that provide the raw material for metagenomic libraries, the size of the libraries that need to be screened, and the diversity of screens needed to assess the small-molecule potential of these libraries, mean that a large commitment of resources to brute-force screening will be needed to help this technology succeed.

Yet the structure of contemporary funding mechanisms makes support for this type of work far less likely today than during the heyday of antibiotics development. Most federally funded, competitive research programs reward hypothesis-driven, rather than exploratory, research.

Hypothesis-driven work is necessary, of course. It has produced staggering advances in our understanding of molecules, cells, and organisms over the last few decades. But it leaves little room for expansive exploratory studies that lead to descriptive information about the diversity of organisms or the chemicals they produce. Proposals involving brute-force, repetitive screening, often do not fare well in the peer-review process, yet it is precisely these types of approaches that are needed to give metagenomics a fair test.

The federal government thus far has only dipped its toes into metagenomics, launching a few, poorly funded initiatives. The National Science Foundation's Microbial Observatories Program, for instance, which supported some of the pioneering work in metagenomics, has an annual budget of $6.4 million. The National Institutes of Health funded its New Methodologies for Natural Products Chemistry initiative with a one-time expenditure of about $2.1 million.

Metagenomics needs, and deserves, a more substantial federal investment — at least another $10 million per year. That's a huge boost in funding, yet it represents just a fraction of what we spend on heart disease. We face a continuously evolving bacterial enemy with ever-less effective medicines, and a pipeline of new drug candidates that is pitifully narrow. When the existing antibiotics cease to work altogether, what then?

We don't know whether metagenomics will yield a treasure trove of antibiotics; optimism is based on only a little data, logical inference, and intuition. One thing is certain, however: Without sufficient aid from federal and private sources, the technology stands no chance of filling the antibiotics void. ⊗

Jo Handelsman (joh@plantpath.wisc.edu)

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Jo Handelsman is a Howard Hughes Medical Institute professor in the department of plant pathology at the University of Wisconsin, Madison. Her research focuses on the genetic and chemical diversity of microorganisms in soil and the role of small molecules in robustness of the microbial community that resides in the midguts of caterpillars.