A report from the American Academy of Microbiology

Systems Microbiology:

Beyond Microbial Genomics

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EXECUTIVE SUMMARY

The American Academy of Microbiology convened a colloquium June 4-6, 2004, in Portland, Oregon, to confer about the scientific promise of systems microbiology. Participants discussed the power of applying a systems approach to the study of biology and to microbiology in particular, specifics about current research efforts, technical bottlenecks, requirements for data acquisition and maintenance, educational needs, and communication issues surrounding the field. A number of recommendations were made for removing barriers to progress in systems microbiology and for improving opportunities in education and collaboration.

Systems biology, as a concept, is not new, but the recent explosion of genomic sequences and related data has revived interest in the field. Systems microbiology, a subset of systems biology, represents a different approach to investigating biological systems. It attempts to examine the emergent properties of microorganisms that arise from the interplay of genes, proteins, other macromolecules, small molecules, organelles, and the environment. It is these interactions, often nonlinear, that lead to the emergent properties of biological systems that are generally not tractable by traditional approaches. As a complement to the long-standing trend toward reductionism, systems microbiology seeks to treat the organism or community as a whole, integrating fundamental biological knowledge with genomics, metabolomics, and other data to create an integrated picture of how a microbial cell or community operates. Systems microbiology promises not only to shed light on the activities of microbes, but will also provide biology the tools and approaches necessary for achieving a better understanding of life and ecosystems.

Microorganisms are ideal candidates for systems biology research because they are relatively easy to manipulate and because they play critical roles in health, environment, agriculture, and energy production. Potential applications of systems microbiology research range from improvements in the management of bacterial infections to the development of commercial-scale microbial hydrogen generation.

A number of technical challenges must be met to realize the potential of systems microbiology. Development of a new, comprehensive systems microbiology database that would be available to the entire research community was identified as the single most critical need. Other challenges include difficulties in measuring single-cell parameters, limitations in identifying and measuring metabolites and other products, the inability to cultivate diverse microbes, limits on data accessibility, computational limitations associated with data integration, the lack of sufficient functional gene annotations, needs for quantitative proteomics, and the inapplicability of current high throughput methods to all areas of systems microbiology. Difficulties have also been encountered in acquiring the necessary data, assuring the quality of that data, and in making data available to the community in a useful format.

Problems with data quality assurance and data availability could be partially offset by launching a dedicated systems microbiology database. To be of greatest value to the field, a database should include systems data from all levels of analysis, including sequences, microarray data, proteomics data, metabolite measurements, data on protein-protein or protein-nucleic interactions, carbohydrate and small RNA profiles, information on cell surface markers, and appropriate supporting data. Regular updates of these databases and adherence to agreed upon data format standards are critical to the success of these resources.

It was recommended that educational requirements for undergraduate and graduate students in microbiology be amended to better prepare the next generation of researchers for the quantitative requirements of applying systems microbiology methods in their work.

Systems microbiology research is too complex to be the sole property of any single academic discipline. The contributions of microbiologists, computer scientists, control theorists, biostatisticians, and others are all required to move the field forward. Since research in systems microbiology demands the contributions of a diverse array of professionals, collaboration across disciplines and national borders should be strongly encouraged by research bodies and funding agencies.

Although the details of systems microbiology research are probably not of interest to the average individual, the potential applications and benefits of these types of investigations should be conveyed to the lay public.

INTRODUCTION

Systems microbiology is changing the way we look at the microbial world. There are as many definitions of "systems microbiology" as there are researchers who engage in it, but the essence of the discipline is this: **systems microbiology seeks to identify how microbial functions evolve and how emergent properties in cells and communities arise from seemingly simple, linear genetic sequences.** The idea of applying systems analysis to the study of biological entities is more than 50 years old, but recent advances in genome sequencing and other high throughput measurements have turned the fanciful imaginings of the past into the ambitious goals of the present.

Since the inception of microbiology, the field has generally embraced reductionism, focusing on increasingly smaller details of microorganisms over time. Systems microbiology complements that trend, seeking to explain the properties that arise from interactions of the smaller parts of an organism or between members of a microbial community. Identifying each of the genes and proteins in an organism (as is often done in reductionist approaches) is analogous to creating a catalog of parts in a car. Just having access to the parts catalog doesn't tell you how to build the car, and it won't reveal the principles and operating mechanisms of the whole vehicle. To proceed, one needs to know how the individual parts interact to convert fuel into electricity and forward motion. Similarly, achieving a real understanding of an organism requires insight into how all the various parts relate to each other and how their dynamic interactions regulate the complex molecular cascades that make life possible. Rather than describing and treating the individual parts of a microbe, systems microbiology treats the microbe or the microbial community as a whole, seeking to understand the interplay of its genes, proteins, and organelles, and how they respond to external stimuli.

Researchers investigating microbes and microbial communities from a systems perspective may use techniques from many fields, including microbiology, biochemistry, genomics, bioinformatics, chemistry, and computational science, among others. However, modeling cellular, organismal, and community dynamics is what sets systems microbiology apart from these other

Systems Microbiology in Action:

Systems Analysis of Photosynthetic Bacteria

The capture of light energy by photosynthetic organisms is the foundation of the biosphere. Photosynthetic plants and microorganisms harvest the sun's energy and convert it into the stuff of life: by splitting water they create the oxygen that sustains the biosphere and by harvesting carbon dioxide and nitrogen gas from the atmosphere they provide the nutrients at the base of the global food chain. Research on photosynthetic bacteria has provided key insights into the critical processes that photosynthetic microbes and plants carry out in support of life on this planet.

Today, systems approaches are being used to analyze the photosynthetic bacterium Rhodobacter sphaeroides. By scrutinizing the patterns of gene expression R. sphaeroides exhibits in the presence of light energy, scientists have been able to identify previously undiscovered genes that are required to carry out photosynthesis. Moreover, genes that have an impact on global climate have been identified by using systems concepts to analyze gene expression in R. sphaeroides' in the presence of greenhouse gases. Genes that degrade toxic compounds and produce renewable biofuels have been discovered in a similar manner. Investigators have also used systems microbiology to catalog the proteins in R. sphaeroides' photosynthetic apparatus, providing new ways to test the functions of many previously uncharacterized components of the light-gathering machinery.

fields. In the systems approach, models of the microbe are constructed at every level, including molecular, subcellular, cellular, and community functions.

Because it encompasses many varied fields of expertise, the work of systems microbiology is, by nature, interdisciplinary and is not the sole property of any single traditional academic discipline.

The larger field of systems biology has been defined by Ideker, et al. (2001), as, *"studying biological systems by systematically perturbing them (biologically, genetically or chemically); monitoring the gene, protein, and informational pathway responses; integrating these data; and ultimately formulating mathematical models that describe the structure of the system and its responses to individual perturbations."* * Systems biology is unique in its effort to identify the components of a living system, understand how those parts fit together, and determine how the parts function as a whole.

The cumulative knowledge produced by decades of reductionist experimentation serves as the foundation upon which systems biology is built. By leveraging the fundamental discoveries of the 19th and 20th centuries, systems research is drawing on the accomplishments of the past to build a more complete knowledge of living things. It is not a substitute for conventional research, but, rather, represents an evolution of biological research building on previous knowledge as well as on recent and future technological developments. In this way, the systems approach adds to the past and current reductionist efforts to understand biology. In the future, the systems approach to addressing biological questions will provide answers to some of the greatest outstanding questions in biology and will provide the means by which we can predict and manipulate the function and evolution of living systems.

WHY SYSTEMS MICROBIOLOGY?

Systems microbiology is a specialization within systems biology, and to derive a parallel definition for systems microbiology only requires changing "studying biological systems" to read "studying microbiological systems" in the Ideker description. However, if systems microbiology is merely a subcategory of systems biology, then why does it deserve particular attention? Can systems microbiology offer lessons about life that inquiries into the biology of plants and animals cannot? The answer is an unqualified "yes." Clearly, studying microbes from a systems perspective can yield long-sought answers to questions about microbes and their roles in human health and the function of the biosphere. But perhaps more significantly, this approach can also lead to the development of the tools and insights that will eventually be applied to the systems analysis of non-microbial life.

Microbial systems are important and ideal models

Bacteria, archaea, eukaryotic microbes, and viruses offer easier access to the workings of biology than do multicellular organisms, a fact that systems microbiology can surely exploit. The technical details of cultivation are undoubtedly less difficult for many microbes, which can be grown in petri dishes, tubes, and flasks, than they are for larger organisms, which require more complex growth conditions and more space. Microbes can also be grown asexually, allowing researchers to study clonal groups of cells, a feat that is often impossible with multicellular life forms. For decades these characteristics have allowed researchers to perform

Systems Microbiology in Action:
Gene Regulatory Networks and Designer Microbes

Systems microbiology is enabling scientists to design novel microbes that can be used to address environmental, agricultural, or medical problems. Researchers are using systems approaches to weave existing data on well-studied organisms into an integrated picture of how microbial cells function. By modeling the metabolic and regulatory networks of common lab inhabitants like Escherichia coli, scientists can now build novel gene circuits that respond to new signals in a predictable way. These "designer microbes" have a number of potential applications, including the degradation of persistent toxic chemicals that would otherwise poison soils and water supplies. Engineered bacterial strains have also been used as microbial factories for generating ethanol (an important biofuel), feed additives, and pharmaceuticals. Microbial production of these materials can be more cost-effective than production by traditional methods.

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* Ideker, et al., 2001. Annu. Rev. Genom. Hum. Genet, 2: 343.

experiments under strictly controlled conditions. Systems microbiology is poised to capitalize on these aspects of microbes in order to provide new insights into their operation and to develop the platforms that can be applied to other living systems.

With the exception of fungi and certain other eukaryotic microbes, microorganisms carry relatively small genomes. The size of microbial genomes has enabled researchers to sequence the entire genetic material of thousands of viruses and hundreds of bacteria and archaea, and many more are sequenced every year. The ability to easily sequence major stretches of microbial genomes is particularly useful in studying the molecular and genetic basis of evolution, a phenomenon that is amenable to an experimental approach in microbial systems but is more difficult to explore in multicellular organisms.

Microbes offer important lessons about all life forms

Although microbes are structurally simpler than larger life forms, they are intricate organisms that collectively harbor most of biochemical diversity on the plant and have a great deal to teach us about life on earth. In many ways, they are more dynamic than the individual cells of multicellular organisms, which exist in relatively protected, controlled environments. Because of their exposure to the stresses and vagaries of life outside a larger life form, microbes are forced to be more adaptable and responsive to their environments than are metazoan cells. These are important features that systems microbiology can tackle and apply to improve our understanding of these abilities in all organisms.

Microbes can offer lessons in the interdependence of biological responses to changing environments. Since they exhibit almost all the critical cellular features of living cells in a relatively simple package, microbes can also enable a predictive understanding of interrelationships across scales, such as the relationships between molecular functions, sub-cellular functions, cellular functions, and so forth. Microbes are good platforms from which to study the emergent properties that come from the interacting parts of all organisms and groups of organisms. Microbes also offer insights into cellular life in multiple contexts, both as individual organisms and as members of a community.

POTENTIAL APPLICATIONS OF SYSTEMS MICROBIOLOGY

The broad aim of systems microbiology is to acquire an understanding of the wiring diagrams of life – to grasp the relationships between the individual components that build an organism or a community. In seeking to move past the basic study of biological components (including molecules, enzymes, microbial species, etc.) and into a synthesis that can be used to predict the future state of biological systems, systems microbiology promises to provide powerful new tools and insights contributing to agricultural, medical, industrial, and environmental innovations.

The practice of **agriculture** stands to benefit in a number of ways from the information gathered through systems microbiology. For example, the procedures surrounding meat production could benefit from improved systems knowledge of microbial causes of cattle, chickens, and swine diseases, the epidemiology of those diseases, and the influences of pathogen transport. Similarly, high-density aquaculture could be improved through a parallel knowledge of the pathogens of fish and shellfish. The beneficial aspects of soil microbes or communities on plant productivity could be explored using systems techniques and could decrease our dependence on agricultural chemicals. Likewise, the impact of microbes on diseases of agricultural crops could be better managed with a systems understanding of the interplay of microbes, plants and pesticide application.

In **medicine**, systems approaches can offer strategies for identifying drug targets, overcoming antibiotic resistance, and managing the emergence of new diseases. A systems understanding of human pathogens will likely enable society to design antibiotics that target the weakest parts or Achilles heel of the organism. Alternatively, systems approaches could identify the appropriate antibiotics for use in multi-drug strategies that target pursue two or more steps simultaneously – an approach that promises to reduce the development of antibiotic resistance. An improved understanding of pathogens from a systems perspective will also facilitate management of hospital acquired infections. Using these methods to study pathogen ecology can offer insights into interventions that will prevent the emergence of new diseases from environmental sources.

Other applications where the predictive abilities of systems microbiology could be put to use include:

- **Novel energy production systems.** The development of commercial microbial hydrogen generation, BIO-batteries, and other technologies for energy production could be optimized by a better understanding of the microbial cell or microbial community as an integrated system.

- **Metabolic engineering.** Existing commercial bioreactors and the products derived from organisms inoculated into them could be optimized through a systems approach.

- **Biocontrol.** The use of ‘helper’ microbes to eliminate or control undesired microbial populations could be made possible through a systems understanding of their interactions in soil and aquatic communities.

- **Pollution and bioremediation.** Water and soil quality management systems could be optimized with systems approaches.

- **Bioterrorism and decontamination.** By identifying key steps in the function of potential bioterrorism agents, systems microbiology can help detect the release and prevent the spread of these microbes.

- **Microbiological detection systems.** Novel, highly specific, and sensitive detection systems and diagnostics can be easily developed using systems methods.

- **Global monitoring.** As the most numerous and diverse life forms on this planet, the activities of microbes and microbial communities can be used as sensitive reporters of local or planetary changes in temperature, greenhouse gases, pollutants, etc.

RESEARCH IN SYSTEMS MICROBIOLOGY

A number of specific issues in systems microbiology research merit discussion, including the scientific fields that should be involved in projects of this type, how systems microbiology can be applied to studying biological regulation and microbial communities, the role of a systems approach in hypothesis-generating research, and how the presence of ”noise” in biological systems will affect progress in systems microbiology.

DISCIPLINES THAT CAN CONTRIBUTE TO SYSTEMS MICROBIOLOGY

What fields can contribute to systems microbiology? Progress in this field needs the contributions of a diverse range of professionals. That is not to say that all problems will require the input of many different fields of expertise, as there are inevitably going to be goals that can be accomplished by individuals and goals that will require a consortium of scientists. Some of the professionals that are needed for these investigations include:

- Microbiologists,
- Biochemists,
- Evolutionary biologists
- Mathematicians,
- Computer scientists,
- Physicists,
- Chemists,
- Control theorists,
- Systems engineers,
- Geochemists,
- Atmospheric chemists,
- Chemical and physical oceanographers,
- Earth scientists, and
- Biostatisticians.

While specialists in each of these fields can make contributions to developing a systems approach to microbiology, interdisciplinary researchers familiar with microbiology will be in an excellent position to advance the field.

REGULATION OF BIOLOGICAL SYSTEMS

Due to the number and complexity of interactions involved in the biological pathways and regulatory networks, progress in understanding these systems has often been made by studying one isolated circuit at a time. As a result, limited progress has been made in describing the interactions between different components of interconnected regulatory networks. Systems microbiology may be the perfect approach for tackling this crucial subject. Using the tools of systems biology, researchers can begin to delve into the basic mechanisms of regulation, uncovering previously unknown types of interactions. Other, broader questions can also be addressed, including whether fundamental design principles exist in biological regulation and whether understanding these design principles offers insights into the in situ behavior of organisms.

Although a number of systems methods for addressing questions about biological pathways and networks already exist, new methods and approaches are needed. Investigators and technology development professionals need to consider which investments of time and money will have the greatest payoff in understanding these fundamental aspects of microbes and other living systems.

APPLYING A SYSTEMS BIOLOGY APPROACH TO MICROBIAL COMMUNITIES

Current and future systems microbiology techniques can provide approaches to understanding the complex properties of microbial communities, their dynamics, and their impacts on natural and human systems. Systems approaches to microbial communities could answer the following fundamental questions: Which species are present? What are they doing? Where are they doing it? What is the environmental impact of the community? And finally, what happens to the community and its impacts in the event of a natural or society-generated disturbance?

As a first step in these investigations, it will be necessary to identify all the members of the community under study as well as all the interactions in which they engage. This is no small feat, and the ability to accomplish this kind of undertaking is likely to require new analytical and computational tools. The systems approach may at first find its greatest success by focusing on relatively simpler communities in which there is little overlap between the niches occupied by different members of the community. Stochastic effects may obscure the picture in communities in which there is a high degree of redundancy.

Spatial organization is another confounding factor in carrying out an analysis, but it may be avoided by studying laboratory communities in liquid suspension. In any event, and whichever community is selected for study using systems analysis, it is likely that single cell biochemical techniques will be needed as one of the key dissecting tools (see **Technical Challenges in Systems Microbiology** section – *Single cell measurements).*

Systems microbiology approaches should avoid treating the sum of all the genomes from a given environment, also known as the metagenome, as a single entity. Researchers should instead develop methods for assembling the individual genomes of the possible thousands of community members. The cell and its genome are critical units of organization in microbial communities and they should not be dissolved in research that hopes to achieve an understanding of these associations.

HYPOTHESIS-GENERATING RESEARCH

By mining high throughput data, scientists can achieve new insights and derive novel hypotheses that would be time-consuming or impossible to develop through traditional reductionist approaches. Many successful examples of applying hypothesis-generating principles exist, including the genome mining work that uncovered motility and chemotaxis genes in Geobacter species. Prior to the genome mining work, these organisms were thought to be non-motile, but following the discovery of motility genes, experiments were implemented that confirmed their motility. Both tactics, hypothesis-generating research and hypothesis-driven research, are required for successful application of systems approaches.

MEASURING NOISE IN BIOLOGICAL SYSTEMS

Noise is a problematic factor in all branches of experimental science, biology included. In biology, some of the difficulty lies in separating measurement noise, the irreproducible quantitative variation due to observational factors, from true biological noise, the irreproducible variation exhibited by biological systems. Moreover, stochastic processes have been shown to drive some biological phenomena, so biological noise cannot be dismissed from data analysis lest a significant underlying process be ignored. Hence, noise is an inescapable part of biological science and must be addressed in investigations that employ systems approaches. The ability to measure noise in a given biological system will depend upon the ability to quantify its relevant features.

TECHNICAL CHALLENGES IN SYSTEMS MICROBIOLOGY

As an evolving field, most of the successes of systems microbiology lie in the future. However, to realize this potential the technical challenges that riddle the path ahead must be acknowledged, managed, and overcome. Technical bottlenecks range from difficulties in identifying and quantifying cellular constituents to limitations in the ability to cultivate diverse microbes or monitor the activity of complex microbial communities. Difficulties also arise in acquiring and cataloging the necessary information and research data and in assuring data quality.

A database of systems microbiology information would facilitate the process of overcoming these difficulties in data acquisition and quality assurance and would most likely prove to be hugely advantageous for the field.

TECHNICAL BOTTLENECKS

Since its inception, progress in microbiology has proceeded in lockstep with advances in technology. This dependence on technology also extends to systems microbiology, and a number of methodological difficulties must be resolved for the field to move forward. Many of these bottlenecks are universal and apply to multiple systems, but others are more system-specific.

Single cell measurements

The inability to measure biochemical parameters in single cells poses a serious restriction on the power of systems microbiology. Average measurements of tens, hundreds, or even millions of cells have been useful to microbiology research, but to better understand the range of variation and the true dynamics of an organism, measurements of activity in single cells will be necessary. Quantitative measures of gene expression, protein levels, metabolites and other cellular constituents are needed to complete the picture of biological systems. It is highly desirable to be able to carry out these measurements non-destructively and in real time. Tracking single cell measurements over time would be particularly helpful in following components that are located at specific points in the cell or on the cell membrane.

Identifying and accurately measuring metabolites

Difficulty in identifying and localizing metabolites and in determining the pool sizes of these components poses another obstacle to applying the systems approach. The sensitivity and spatial resolution of the currently available methods need to be improved in order to model the behavior of metabolites with the proteins that use these compounds as substrates, products, or ligands. Greater sensitivity and resolution would also establish tighter links between observations of community structure and function.

Cultivation

The inability to isolate and cultivate many types of microbes has long limited the range of organisms that are available for analysis. Although the vast majority of microbes resist cultivation by traditional methods, it has been proposed that many more strains would yield to cultivation efforts if novel, imaginative approaches were used. Systems microbiology would benefit greatly from renewed efforts to cultivate diverse strains or consortia of microbes from different environments, since the ability to study individual strains under lab conditions is often a key to experimentation.

Data accessibility

Enormous quantities of biological data have been accumulated over the years, including genome sequences, annotations, biochemical information, microarray profiles, and other types of information. These data could serve as an invaluable resource for developing a systems understanding of microbes, however, many important data sets are unavailable to public databases and others are not amenable to storage or comparison in a database format (video images etc.). Ideally, these data sets should be stored in searchable, cross-referenced databases that allow researchers ready access to information pertaining to their individual research

OTHER TECHNICAL BOTTLENECKS IN SYSTEMS MICROBIOLOGY INCLUDE:

- **Difficulties in sequencing DNA from microbial communities.**
- **Difficulties in analyzing microbial communities in situ.**
- **Generally low analytical sensitivity for microbes that are hard to culture or slow growing.**
- **Limited facilities for controlled cultivation of microbes.**
- **Lack of a coordinated repository for depositing analyzed microbial cultures or communities.**
- **Poor infrastructure available for imaging,**
- **Difficulties in managing and archiving large electronic files, particularly images.**
- **The lack of available methods to visualize the presence and activity of proteins or protein complexes.**
- **The dearth of user-friendly bioinformatics applications.**
- **The lack of modeling paradigms that can accommodate broad applications in time and spatial dimensions.**
- **The lack of a collaboratory informatics infrastructure that biologists can use, and**
- **The inability to measure biological responses that are close to the current baseline of detection.**

efforts. For example, with new microarray data in hand, a researcher could access a relevant database to discover the location and context of a gene of interest, its place on a metabolic map of that organism, its relationship with or within regulatory networks, and related genes or pathways in other organisms. In order to make the best use of archival data and to move forward in the field, data of use in systems microbiology must be publicly accessible and encoded in a format that can easily be cross referenced. (Further discussion of this and other requirements for data formatting and databases are included in the section titled **A Systems Microbiology Database**.) The quantity of biological data is expanding daily, and the current state of disorder will only worsen if steps are not taken to organize data relevant to systems microbiology into a useful, convenient resource.

Computational limitations

Even given the unprecedented advancements in computing power that have been achieved over the past decade, certain computational limitations still impose restrictions on the type and dimension of systems modeling that can be accomplished. Although a "bottoms-up" modeling approach, in which the activity of a system is simulated from the known or suspected activity of its components, may be too complicated to tackle for a number of years to come, greater computing power could enable a top-down microbial modeling approach in the near future. In top-down models, measures of the end products of the system are used to predict the activity of the system. In these models, it may be most appropriate to focus on the role of those proteins known as "master regulators" (which direct the responses of the cell and the cell cycle) and the top-down regulatory architecture. The development of this type of model could enable the subsequent modeling of collections of interacting organisms in simple consortia or complex communities.

Annotation and functional characterization of genes

Gaining an understanding of the function of a gene product poses yet another barrier to progress in systems microbiology. Annotation and the comprehensive functional characterization of proteins or RNA molecules remains difficult, error-prone processes, but systems microbiology relies heavily on a thorough understanding of the functions of gene products. Making more and better annotation and functional information available to systems researchers would enable improved gene and gene function predictions (in newly sequenced organisms), and allow evaluation of context-dependent expression and function.

Proteomics

In order to promote the success of systems microbiology, the development of new proteomics approaches needs to continue. To date, proteomics (the study of the full complement of proteins in the genome of an organism) has been slow to generate the types of quantitative data needed for use in systems applications.

High-throughput technologies

Although some high-throughput technologies are relatively mature – such as the use of microarrays for gene expression measurements – many of the data needed for systems microbiology currently cannot be obtained using high-throughput techniques, presenting a serious limitation on the rate at which the field can proceed. Additional high-throughput technologies are needed. These include, but are not limited to, the production and characterization of the vast array of proteins encoded in microbial genomes, identifying and characterizing the interactions among the various proteins and other macromolecules, determining the type and concentration of intracellular metabolites and extracellular signal molecules at a given cell state, accurately monitoring the presence of key regulatory RNA molecules, and determining the suite of surface components that can often drive microbe-microbe and microbe-surface interactions in biofilms.

INFORMATION AND DATA GAPS

While there are idiosyncratic differences in the type of type of information that needs to be gathered to characterize different biological systems, certain general requirements can be identified that are needed to characterize any given system. This information includes the "parts list": a comprehensive accounting of the components of the system at many levels of organization, including the molecular, subcellular, taxonomic, and environmental levels. However, in many cases the entire list of components is not always needed to develop a thorough understanding of the behavior of a biological system. Even in the absence of complete knowledge, the systems approach can still work well.

There is also a need to obtain "global information," or comprehensive measurements of cellular components, for each system under study. This may include, for example, the types and levels of regulation that occur in the system or the types, activities, locations and fluxes of cellular proteins, nucleic acids, metabolites, carbohydrates, and other cellular constituents. There is also a need to understand how an organism lives in its natural state. The evolutionary history of the system across multiple temporal scales is also necessary.

Once the systems approach begins to yield results, the information gathered is likely to feed back and indicate the most critical data that are needed to move forward in the analysis.

Needed data

A great deal of data that may be of use in systems microbiology has already been generated. Genome sequences from cultivated and non-cultivated organisms are a considerable resource for this effort, as are the gene inventories that have characterized the genetic diversity of a broad range of different environments. Other information, including microarray data, exists but is not readily available to the research community in the form of an easily accessible public database. There is also concern regarding the relative quality of these data.

Strategic decisions are needed in collecting the additional data necessary to pursue systems microbiology. It may be advisable, for example, in the effort to define microbial systems, to weigh the relative advantages of studying one microbe under multiple different conditions rather than studying many different strains under one condition.

Continued genome **sequencing** is crucial to the efforts of systems biologists. In particular, more sequences are needed from more organisms derived from a greater range of habitats. Dense sequencing of certain branches of the bacterial and archaeal phylogenetic trees can provide important information about the mechanisms of cellular differentiation and evolution. For example, many closely-related Proteobacteria have widely diverse morphologies, niches, and metabolic systems. Although certain regulatory molecules are highly conserved in this branch of the tree, they have been found to control widely different functions in each of the different species. Systems analysis of genome sequences and organization could determine how these control systems evolved.

Genome sequences can only get systems biology so far, however. It is also critical to obtain more data on the **functions of the proteins or nucleic acids** encoded by these sequences. Scientists currently rely too heavily on homology mapping in inferring the functions of proteins and protein domains. Homology mapping involves comparing a given protein sequence to the sequences of proteins that have been characterized previously. Unfortunately, not all protein functions assigned in public databases are accurate, creating a situation in which the functions of many proteins remain unknown or are possibly misconstrued. Such cases of misassignment can hamper advances in the field. The results of sequence homology analyses can also be vague; often, proteins can only be given nonspecific descriptions, like "hypothetical kinase" or "transmembrane protein". Moreover, many genes that are discovered today have no homologs among the functionally described genes and cannot even be assigned a tentative function.

Accurate gene annotation will be vital to progress in systems microbiology. In many cases, considerable experimentation is necessary to confirm the inferences of gene function that are suggested by homology mapping and other sequence-based approaches. Experimental evidence about at least one protein in a family of proteins would prove extremely helpful to systems efforts. Methods for updating annotations in public databases are also needed so that researchers can be made aware of new insights on the function of related gene products.

Accurate quantitative information on a variety of biological parameters is needed to model the function of biological systems. Particularly necessary are data that can describe a system sufficiently to allow treatment of that system as a black box component of larger systems. These data can include flux measurements, association characteristics (between components and individuals), three-dimensional architecture, context dependent functional measurements (including locational dependency of enzymatic functions), physical and chemical characteristics, and the spatial-temporal relationships between events in the system under study.

It has long been known that results of experiments performed in vivo differ from those performed in vitro and that laboratory type strains differ from wild type isolates. More extensive **sampling of microbes in their innate habitats**, *in vivo* or *in situ*, is necessary to characterize microbial systems without the biases incurred by growth in the laboratory environment. Also, natural isolates change, sometime dramatically, with extended culture in the laboratory; genome rearrangements, loss of virulence, and loss of complexity in biological processes have both been noted after lengthy culture. For this reason, lab cultures may not provide appropriate models for natural biological systems. As genome-based modeling of microbes in their natural state is improved and metagenomic data are made available, it may become easier to estimate the activities of those microbes that cannot be cultivated or whose activities are altered in cultivation, including their environmental activity and their requirements for growth.

Other types of data that are needed for systems microbiology efforts to proceed include:

- Data that indicate spatiotemporal relationships between system components,

- Data on community architecture,
- Data that can reveal design principles and biological networks,
- Data that expand the phenotypic characterizations of cells and communities,
- Data on the natural variability of microbial systems, and
- Data on cell cycle or spatial events.

ACQUIRING INFORMATION AND DATA

Difficulties have arisen in collecting the data necessary for systems microbiology investigations. Existing disparities in formatting and data standardization between databases, for example, can hinder the ability of researchers trying to acquire information from prior investigations. Often, the necessary information is stored in a database or in the literature, but the representations or archive layout can prohibit easy access. These data are seldom subjected to a quality assessment, and researchers access these resources at their peril. Also, the representation of biological data in these databases often reflects a cultural gap in understanding between the computational scientists who design these databases and the biologists who use the data. In general, there is not enough support for the databases and other information sources critical to the advancement of systems microbiology.

The documentation of metabolic pathways poses a particular problem; there is a pressing need for standardization in these data sets. Scientific societies, journals and/or funding agencies should move to set standards for formatting of metabolic and regulatory pathway information. These formatting guidelines could apply to the type and organization of metabolic pathway information provided in research publications and submitted to public databases. It is also crucial that these data are stored in a "searchabl" way that allows researchers to explore pathways through a database interface.

Due to the high costs of many of the technologies necessary for carrying out the work of systems microbiology, it may be advisable to develop central "locations of excellence" to serve as a resource for the scientific community. High resolution microscopy, other imaging or modeling technologies, and high throughput data production facilities, for example, which are too costly for many labs or universities to provide, could be housed in collective centers to the benefit of researchers and the field in general.

ASSURING DATA QUALITY

The problem of how to ensure and validate the quality of data available in public databases has become more and more challenging with the genomics data explosion of recent years. Physics and other fields have grappled with large data sets in the past and the manner in which the dilemma was solved in those areas may offer lessons to biology today. The contributions of computer scientists and information specialists will be crucial in resolving the issue of information management, but it is important that biologists work closely with these professionals to ensure the utility of the information systems that are developed.

Genomic data should not be treated as static; mechanisms need to be put into place to continuously update the sequence and annotation data available in public databases. One serious impediment to maintaining data timeliness is the inability or reluctance of most scientists to commit to the long-term upkeep of their publicly available data. When a student graduates, or the funding for a project is exhausted, the impetus to continue updating and actively curating small databases is often lost.

The manner in which genomics and other critical data are generated should be standardized and reported in both the archival databases and publications. For example, microarray data are more useful only if the details of the experimental conditions and analyses are recorded and documented in an accessible format. This type of information must be included with genomics data sets to ensure the results are interpreted properly.

Quality assurance and reproducibility standards for systems microbiology data that are consistent across all the relevant journals and agencies need to be established. Once proper standards are developed they could be implemented through peer review and incentives from funding agencies.

In applying for funding, researchers are strongly advised to allot money and personnel time to quality assurance tasks.

MAKING SYSTEMS DATA APPLICABLE AND AVAILABLE

Efforts to standardize experiments, analyses, and biological materials across microbiological fields will aid in efforts to develop the field of systems microbiology. For example, defined cultivation practices can be standardized to enable the reproducibility of experiments from lab to lab. The results from experiments carried out under such standard conditions can be integrated across many labs in a coherent framework. In addition, the selection of model organisms or model communities for particular broad areas of research would help to standardize the collection of information in a manner that would be useful to a systems approach. Ideally, model organisms would be selected to represent different evolutionary histories so that data obtained from one phylogenetic branch could be applied to other lineages to determine the biological consistency and universality of particular theories.

It is recommended that a commission on data exchange be established to determine standards for data analysis that would promote exchange of data resources. Finally, a reference microorganism repository with original samples and libraries as well as information on the field sites and environments from which the microbes were derived would also enable researchers from different labs to build effectively on the work of others.

Properly designed databases are pivotal in making the data of systems microbiology widely available. A general standard should be instituted by the professional societies and scholarly journals that requires data to be submitted to an appropriate public database.

A Proposal for a Systems Microbiology Database

How can researchers in systems microbiology, as a community, make the most out of the data that has already been collected? What is the best way to ensure that data from one system can be applied to learn something about other systems? The key to optimizing the use of systems microbiology data lies in sharing quality data openly. The value of a given investigation in systems microbiology is trivialized if the resulting data are not made available to the scientific community in a standardized and accessible format.

Currently, however, protocols for ensuring that quality systems microbiology data are widely disseminated among researchers are absent and, as a result, data are not effectively shared among researchers. Microarray data, for example, are scattered among numerous different repositories, personal websites, and often partly reported in publications and there are no mandatory standards or consistency in their analysis. Moreover, there is no unified way in which to query these and other data. The success of systems microbiology will be limited unless the data of past and present is shared promptly and structured in a way that it can be used effectively. To accomplish these twin goals of dissemination and organization, it is recommended that a central, curated database for systems microbiology be established.

A number of unconnected data resources are available to researchers in systems microbiology, but they are inadequate to address the needs of the field. Databases of raw data and personal data usually lack a quality control framework. Other databases house data that are out of date, are not indexed or searchable, or are not available to the research community at large. A new database is clearly needed. Assembling a database for use by the systems microbiology community represents a central challenge for the field and for systems biology in general.

To be effective, a systems microbiology database should be all-inclusive, searchable, current, and authoritative. The accumulated data should include many levels of information from the different analytical and experimental techniques relevant to the field. To accomplish this goal, a coordinated effort will be required on the part of researchers and those responsible for maintaining the existing data sets and databases to integrate the available information into a logical configuration. This configuration should be easily queried and searched – capabilities that would facilitate the optimal use of the data resources. Efforts to ensure that the data remains up to date would be critical to the success of the database. Also critical are the development of data formatting standards that will streamline submission, searching and retrieval of data. Format standardization must be made a high priority for the field. It is critical that biologists, as the end-users be the driving force behind the design and construction of the database and the contributions of scientists from all nations should be welcomed to the database effort. A database that demands quality and accommodates data from all quarters could serve as the "gold standard" source of information for the field.

The appropriate dimensions of the database are open to debate. Smaller databases that focus on single organisms, for example, may better capture the attention and efforts of professionals who are invested in a particular system. As a result, small databases may be easier to maintain and keep up to date. On the other hand, a larger database could be more efficient and cost effective with respect to administration and hosting issues.

Systems microbiology data for the database

Data from all levels of analysis in systems microbiology should be targeted for inclusion in the database. These include (but are not limited to) the data associated with genomics and other techniques: sequence data, microarray data, proteomics data, data from metabolite studies, data on protein-protein interactions, data on protein-nucleic acid complexes, carbohydrate profiles and profiles of cell surface markers as well as data from previous publications on traditional biochemical, genetic or physiological analyses. Supporting data could also be included. For example, detailed information on the conditions used for cell growth, links to relevant information on the genes and proteins of well-studied organisms, imaging data, and the results of biochemical and physiology analyses would be very useful to researchers. That said, the amount of raw data involved in systems microbiology investigations is staggering, and not all of it can be included in a single database. Thus, it is likely that different data sets will have to be included selectively and linked for the investigator to easily access as needed. The contributions of computer scientists and information engineers will likely be required in finding the solutions to managing and storing these large data sets.

The storage and dissemination of genomics data is a particular need in the systems community today. Researchers need access to primary array data if the lessons learned in a given experiment are to be applied to other systems. Primary microarray, proteomics, and metabolomics data and higher-level analyses should be included in the database.

Naturally, a set of quality standards and rules for cataloguing information will need to be conceived for the database.

Updating the database

The systems microbiology database should be updated regularly. The utility of any data resource in biology diminishes if the information is not kept up to date in a timely fashion. A number of existing biological databases have shown how quickly a useful resource can become obsolete if it is neglected. Mechanisms for easy, continual updating of a database of this kind, either automated or investigator-driven, are currently lacking, however, and the development of an appropriate methodology poses a serious obstacle.

Conventions can change rapidly and, oftentimes, provisionally, in the field of microbiology. Genus and species names, for example, may be revised many times over a span of only a few years, wreaking havoc in finding and curating appropriate information in databases and scholarly publications. Gene numbering can also change, sometimes from day to day during the course of a sequencing project. The database will need to be updated with respect to new species designations and gene numbering, but it should be carried out in a rational way that minimizes confusion for database users.

EDUCATION, TRAINING, AND COLLABORATION IN SYSTEMS MICROBIOLOGY

Systems microbiology represents a new and powerful way of thinking that was necessitated by the ever-increasing volumes of biological data becoming available and the comprehensive nature of genome sequencing, which provides exhaustive information on the genetic contents of cells and organisms. As high-throughput technologies and access to large data sets accelerate the pace of discovery the need for a systems approach to interpret and exploit these volumes of data becomes increasingly apparent. This new point of view needs to be reflected in our approach to education and training in microbiology at the undergraduate and graduate level. Of critical concern is the encouragement and training of professionals who are interested in learning how to integrate complex biological information using a systems approach.

PREPARING MICROBIOLOGY STUDENTS TO USE SYSTEMS APPROACHES

System biology holds great promise for the future of microbiology. Considering the power of these approaches, microbiologists should be trained with an eye to the skills that are necessary to understand and apply systems concepts to microbiological problems. To prepare them for systems microbiology applications, the core curriculum for microbiology students should include:

- Microbiology,
- Quantitative biology and mathematics,
- Biostatistics,
- Biochemistry,
- Physiology, and
- Modeling of biological systems.

Quantitative training for undergraduates

For a number of reasons, undergraduate curricula in the biological sciences have largely excluded intensive quantitative training. For most students, this limits their access to the fields of bioinformatics, computation, and statistics, making them ill-prepared for the rigorous mathematical and statistical requirements of applying systems techniques. Training for biology students in mathematics must be improved and expanded to develop a new crop of numerically literate microbiologists able to meet the challenges of systems microbiology.

Although quantitative training should be extended, systems biologists do not need the same skills as a typical mathematician. Math for biologists should be couched in the language of biology to emphasize the potential applications of math in the field. Specially designed courses in applied mathematics that include a laboratory section to demonstrate the principles discussed in class would be particularly suitable for biologists. Other novel approaches to training include the use of computer-based mathematics programs like Mathematica or statistical programs like Statistica, which may enable students to apply sophisticated math and statistics to biology with less training than would otherwise be required. These approaches can greatly facilitate calculations but they are not a substitute for understanding. Systems biologists need to understand the underlying assumptions of different statistical methods, to know when it is appropriate to apply a specific test.

Summer institutes for undergraduates and graduate students

Summer educational opportunities could be made available to microbiology students to provide intense training in systems microbiology to a select few. These courses could cover such topics as mathematics, computer programming, or systems microbiology in general. The aforementioned centers of excellence in systems microbiology would be natural sites for hosting such institutes.

INSTRUMENTATION TRAINING

Progress in biology is paced by instrumentation; steps forward are enabled by new technologies, and the field can be stymied if new tools are not made available or are unused. Broader training in the high throughput techniques and new analytical, microscopic and modeling instrumentation associated with systems microbiology is highly recommended so that more microbiologists can take advantage of the most current technological advances. In their training, students should be exposed to a number of techniques and approaches so that they can learn to choose appropriate methods to address a given hypothesis.

It is imperative for biologists to communicate with the physicists and engineers who design and construct instrumentation for use in systems microbiology. Oftentimes, the different vocabularies of biology and physical science can stand in the way of effective communication between these professionals. Engineers and physicists who develop biology instrumentation need to be exposed to more biology training to facilitate the necessary collaborations and design the appropriate user-friendly devices to support research activities.

COLLABORATION IN SYSTEMS MICROBIOLOGY

Collaboration is a key ingredient for success in systems microbiology. However, the details of collaboration, including communication between distant institutions, questions of where to situate collaborating scientists, student co-mentoring, co-authorship, and issues to do with funding, tenure or promotion can hamper effective cooperation and career development of involved researchers. These challenges must be dealt with to enable effective interdisciplinary research and collaboration.

Encouraging new collaborations

Steps must be taken to encourage collaborations among the microbiologists, biochemists, computer scientists, engineers, and others who can contribute to studies in systems microbiology. As in other arenas, the best incentive for stimulating collaboration in systems microbiology is a monetary incentive. **Additional funding** is needed to establish programs that include incubator funds, start-up funds, planning grants or supplements to traditional investigator-driven awards in order to facilitate the process of bringing disparate groups of professionals together. The systems microbiology approach can also be encouraged through funding a meeting or laboratory course of interested scientists that could take the approximate form of a Gordon Conference or Specialized Laboratory Course (in which roughly 50 participants hold poster sessions, attend lectures or perform "experiments" in a live-in setting). Ensuring access to funding for students and post-doctoral associates who engage in systems microbiology is also central to making progress in the field. Support for collaboration between academic and industrial interests can yield fruitful collaborations in systems microbiology provided proper controls are put in place to ensure the timely publication of results.

Special attention should be given to choosing how peer review is carried out in evaluating the merit of systems microbiology proposals as the breadth of these proposals may exceed the expertise of individual assessors. It may be necessary do develop special panels to evaluate these proposals.

International collaborations

Researchers in systems microbiology should take full advantage of opportunities to engage in international collaboration. The scientific community involved in this particular topic is extremely cosmopolitan, and researchers from different nations have their own traditional strengths to lend to a given project. In Europe, for example, researchers in Germany and the Netherlands are known for their work in physiological diversity, Poland and Hungary have strong credentials in marrying biology to math and physics, and Romanian scientists have maintained expertise in histological techniques that can be applied to solving systems microbiology questions.

International work is also justified in light of the global nature of the problems facing science today. SARS and other infectious diseases readily cross international borders, over fishing and water quality are problems that must be managed by cooperative teams of nations, and climate change threatens every ecosystem on the planet. Finally, the equipment necessary for carrying out the work of systems microbiology can be extremely expensive, putting it out of the reach of many research groups or individual countries. Collaboration across borders can allow scientists to take advantage of cutting-edge technologies without having to duplicate costly equipment.

International collaboration is not always easy to accomplish, however. A number of significant logistical obstacles stand in the way of effective research. Arranging and funding meetings among international groups of collaborators can be difficult, as can the coordination of funding for collaborative research from grant programs in different countries. Efforts to bring collaborators together from distant institutions would benefit from implementing the newest technologies available in web-based communications. Such systems provide a cost-effective way to hold collaborator meetings and host seminars, and could serve as an important complement to face-to-face communications. Microbiology professional associations from all nations should work in partnership to promote collaborations and to advance the goals of systems microbiology.

COMMUNICATING SYSTEMS MICROBIOLOGY TO THE PUBLIC

Systems microbiology is a complex field that is not readily accessible to or understood by the public, but it will certainly bring great benefits to society. Because of the long-range importance of systems approaches, scientists must make a special effort to educate the public about the benefits of investing in systems research.

One lesson to drive home to the public is that the benefits of cutting edge research, including systems microbiology, are eventually felt by ordinary citizens. The space program, for example, brought the world a number of technological advancements, including the pocket calculator and the home computer. The same will be true of investments in systems microbiology; this research will inevitably benefit the public. The public should also be made aware of the necessity of understanding natural phenomena in order to properly manage these systems when something goes awry. This goal could be achieved by encouraging or requiring researchers receiving public funds to engage in outreach efforts or by encouraging professional societies, institutions or supplementary grant programs to provide financial support for disseminating systems microbiology messages to the public.

The applications of systems microbiology are numerous, but a few applications are more relatable to non-scientists than others. The impacts of systems microbiology on biomedical research, and hence, on human health, should be conveyed to the public, as should the import of the field in managing the health of the global ecosystem. The impacts of the field on managing bacteria, increasing existing or creating new beneficial activities in microbes, and controlling the hazards of agricultural antibiotic use should be communicated as well.

MODES OF COMMUNICATION

A number of modes of communication are open to those interested in conveying systems microbiology lessons to non-scientists. For example, commercial space on television would be an effective, albeit expensive, vehicle for communicating with the public. Educational programs that center on interviews with key scientists in the field could be recorded on DVD and sold to schools for use in the classroom, or targeted for use by public radio and television. Children's or young adult books that convey the excitement of microbiology to the lay population could be a useful vehicle, especially when one considers the scientifically literacy of the average citizen. Microbiology could also be integrated into high school curricula; experimentation with microbial systems can offer relatively low-cost, high-impact lessons for young people. A program that encourages graduate students in microbiology to reach out to the community could establish a long-term commitment to conveying the importance and excitement of microbiology research to the public.

THE ROLE OF THE SCIENTIFIC COMMUNITY

Communication between the scientific community and the general public needs improvement. Scientists can be ineffective communicators when it comes to reaching a lay audience. Hence, a greater emphasis on coursework in writing and presentation is recommended for microbiology students. Also, scientists and the relevant scientific societies need to be more proactive in conveying important science to writers and journalists.

RECOMMENDATIONS

- Systems microbiology requires a balance between breadth and depth of knowledge in the professionals engaged in collaborative research. It is necessary for each of the specialists involved to be knowledgeable in their own field, but they must also have sufficient knowledge of the other areas of the project to contribute fully. Academic and research programs should be aware of these issues as they prepare professionals to function as leaders in their field. Mathematics and computational training for students of microbiology must also be improved and expanded to encourage the development of the numerical skills necessary to meet the challenges of systems approaches.

- A centralized database for systems microbiology data is critical. The ability to use archival data will be necessary to realize the full potential of systems microbiology research.

- The database should enables rapid submission and retrieval of high quality data in standardized formats. This database should include: annotated genome sequence data, metabolic pathway data, microarray data, proteomics data, data from metabolite studies, data on protein-protein interactions, data on protein-nucleic acid complexes, carbohydrate profiles, and profiles of cell surface markers.

- The database should be structured so that researchers studying one microbial system can access parallel information on other organisms or communities.

- Systems and guidelines need to be developed so that existing and new data are made publicly accessible and available in formats that allow easy cross-reference.

- Efficient mechanisms to update publicly available sequence and annotation data should be established.

A number of additional recommendation were made that would significantly enhance our ability to understand microorganisms and microbial communities at the systems level. The power of systems microbiology is seriously limited by the inability to measure biochemical parameters in single cells, and technologies to enable this capability should be a high priority. Average measurements of populations of cells are sufficient for some applications, but the ability to measure expression, protein levels, and metabolites in individual cells is needed to complete the picture of biological systems. New analytical techniques and approaches that can provide data at the appropriate level of resolution need to be developed.

- Systems microbiology relies heavily on genomic sequences; more sequences are needed from more diverse organisms and habitats to ensure progress in the field. Continued progress in the functional annotation of the proteins encoded by genome sequences is also crucial to the success of systems microbiology. Experimental evidence on the function of at least one protein in each family of proteins would prove extremely helpful to systems efforts and should be a high priority. Microbes are the most abundant organisms on this planet and they have immense impacts on ecological, agricultural, animal and human ecosystems. More extensive in vivo and in situ sampling and study of microbes is necessary if systems microbiology is to accurately characterize microbial systems that are unaffected by the physical and chemical forces of the lab environment.

- New collaborations should be fostered among international scientists and across scientific disciplines.

- The benefits of systems microbiology should be communicated to the public through innovative and creative means.

- New educational programs should be established to prepare students for the advances in microbiology through studies in systems microbiology. ■