

Genomics and Bacterial Metabolism

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Abstract

The field of bacterial metabolism and physiology is arguably the oldest in microbiology. Much of our understanding of biological processes and molecular paradigms has its roots in early studies of prokaryotic physiology. After a period of declining interest in metabolic studies (prompted by the resurgence of molecular techniques), genomic technologies are revitalizing the study of bacterial metabolism and physiology. These new technologies bring a means to approach metabolic questions with a global perspective. When used in combination with classical and molecular techniques, emerging global technologies will make it feasible to understand the complex integration of metabolic processes that result in an efficient physiology. At the same time, without increased computational capabilities, the massive amounts of data generated by these technologies threaten to overwhelm, rather than facilitate, this work. For genomic technologies to reach their potential for increasing our understanding of bacterial metabolism, microbiologists must become more collaborative and multidisciplinary than at any time in our history.

Introduction

At the core of any living cell is metabolism, the sum total of all the biochemical processes contributing to cell function. Coordination of these processes results in the physiology we associate with each organism, from bacteria to humans. The field of bacterial metabolism and physiology is arguably the oldest in microbiology. Through the years investigators have recognized the need to understand the fundamental principles of life processes and realized the advantages of using prokaryotic cells to achieve this. The field of microbial physiology has changed through the years. With the advent of techniques that allowed researchers to focus more on molecular details of gene structure, organization and expression, research and interest in classical metabolism and physiology declined. Knowledge of complete genome sequences and the ability to visualize the transcription of entire genomes have brought a renewed appreciation of the need to understand the physiology and metabolism of a bacterial cell. Thus a new generation of microbial physiologists is emerging; those with genetic, biochemical, molecular, and genomic tools in their hands. Without a

doubt, the application of this powerful combination of approaches to the study of bacterial metabolism and physiology will result in impressive strides in our understanding of basic metabolic processes and their integration. While newly developed technologies will facilitate progress in understanding metabolism, it is critical to keep in mind that the classical approaches, and rigorous definition of biochemical function must not be lost in the mix.

Genomic Techniques Encourage Global Thinking

Since the term was coined by Thomas Roderick in 1987, the field of genomics has undergone extensive growth. Initial skepticism from the scientific community about the validity and reproducibility of global techniques (*e.g.*, expression arrays) has largely been replaced by an appreciation for the value of the data that can be generated. This appreciation remains appropriately tempered by a recognition of the limitations of these techniques. Commentaries and critiques continue to force the field to reassess techniques, approaches, conclusions, and goals (Downs and Escalante-Semerena, 2000; Gronow and Brade, 2001; Perego and Hoch, 2001; Zhou and Miller, 2002). The success of this field can be measured by the impact it has had on investigators in all biological disciplines. The flood of press and data generated by global technologies has facilitated, and in fact encouraged, investigators to realize that each subject they study is also a component of a larger complex system. This realization has brought the field of microbial physiology full cycle. Classical microbial physiologists considered the contributions of a whole system, whether it was a cell or a population of cells, when they measured properties of carbon utilization, nitrogen fixation, etc. The advent of molecular biology brought a new opportunity to understand molecular details of components within the cell. The premise that a detailed knowledge of the components would provide an understanding of the whole, encouraged decades of reductionist studies that have produced a solid understanding of a large number of molecular processes. Now, with the advent/implementation of global technologies, the pendulum is swinging back, and research characterized by a global perspective is increasing. In the same way classical physiology was enhanced by molecular techniques, emerging genomic technologies provide another dimension to the study of bacterial metabolism/physiology. Data from global analyses (expression profiles, protein profiles, etc.) provide a framework to identify correlations and generate hypotheses. The pursuit and rigorous testing of these hypotheses, not the accumulation of data, will characterize the success of metabolic studies in the genomics era.

Technology has advanced to the point that expression profiles, protein profiles, and other global patterns, are routine enough to be used as a "global phenotype". Reports of global analyses (particularly expression arrays) are

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increasing exponentially in the literature. From these data new hypotheses about the role of a sequence, the function of a protein or the extent of a regulon, are being generated. These technologies force a broad perspective that previous researchers did not have, and thus provide an additional context in which metabolic dogmas can be tested. When the data analyses from global technologies generate new hypotheses and force old dogmas to be questioned, it provides a healthy infusion into the field of bacterial metabolism and physiology (Gonzalez *et al.*, 2002; Rhodius *et al.*, 2002; Wassarman *et al.*, 2001).

For all the potential of approaches using global techniques, caution must be taken with their use. A tenet long held by biologists is that genes expressed together are utilized together. While a valid hypothesis, this tenet is not a basis to assign function. The number of expression profiles being reported is becoming overwhelming. Among these are global data obtained from numerous bacteria, different growth conditions, and different genetic backgrounds. They represent regulation of gene expression due to nutrient deprivation, antibiotic treatment, and carefully chosen genetic defects (Karlin *et al.*, 2001; Ng *et al.*, 2003; Tao *et al.*, 1999; Weber and Jung, 2002; Zheng *et al.*, 2001). Without question these studies have opened new and exciting directions for research in our efforts to understand gene function and the complexities of bacterial metabolism. The sensitivity of these techniques to small differences in expression levels, and the fact that populations are being analyzed, make it critical that investigators are conscious of subtle changes that might occur in growth conditions or sample preparation. Without careful controls, investigators can misinterpret the cause (and thus the relevance) of the variations in expression detected. In addition, a change in transcript level can reflect either direct or indirect effects of the parameter being addressed. Further, the level of transcription does not always correlate with the level of protein, much less functional protein. For this reason, the global analysis of protein (*i.e.*, proteomics) is gaining popularity (Jungblut *et al.*, 2001).

Global analyses of gene expression are being preformed by investigators from a variety of disciplines with a number of goals. While this use reflects the broad value of these technologies, this diversity and rapid growth make it more difficult to enforce standards in the technology. If genomic technologies are to be used efficiently, efforts must be made to ensure the data from these studies are considered in the context of existing metabolic literature. Relevant research results from the past should be identified and addressed in models that are presented based on genomic data. Maintaining this inclusion becomes difficult as researchers from multiple disciplines, that may not be aware of this literature, employ these technologies. Conclusions about metabolism based on genomic technologies that appear to violate a vast literature, need to be recognized and discussed. When considered in isolation, expression array data is of limited use, but in combination with knowledge from decades of metabolic and regulatory studies, it can generate a deeper understanding of bacterial metabolism and physiology. If the extensive literature from previous metabolic studies is

not incorporated into the thinking supported by new technologies, investigators will too often end up "rediscovering the wheel".

The amount of literature one must be aware of to ensure identifying all processes that may impinge on a single pathway in the cell is staggering. A concern with global approaches is that so much data can be generated so quickly, it will be nearly impossible for an investigator to identify and incorporate the literature that he/she should be aware of in interpreting these data. Collaboration and extensive communication between researchers versed in genomic approaches and those with a knowledge of classical physiology and metabolism, in addition to advances in data storage/analysis, will help address this concern.

Model Organisms-Extending the Paradigms

Results from genome sequencing efforts have emphasized that metabolic processes are conserved in diverse living organisms. These results have led to approaches to reconstruct metabolism in diverse organisms *in silico* (Castresana, 2001; Mittenhuber, 2001; Schilling *et al.*, 2002). Perhaps more importantly, this realization has reinvigorated metabolic research in prokaryotes and validated the study of model microorganisms as a means to define metabolic paradigms. In the case of metabolic analyses, one requirement of a model organism is that *in vivo* analyses are possible. Numerous reports appear every month, of work in model organisms (*e.g.*, *E. coli*, *S. enterica*, *B. subtilis*) that has been prompted, facilitated, and/or justified by comparative genomic analyses. Frequently one reads about a locus and/or phenomenon identified in a non-model organism and investigators are quickly drawn to the system in *E. coli* (or other model organism) due to technical ease, and the vast metabolic knowledge base in this bacterium. An example of this scenario is the study of Fe-S cluster biogenesis, a field that has undergone explosive growth in the last ten years. Work by Dean and others on nitrogenase in *A. vinelandii* identified gene products (NifSUA) required for the formation of metal clusters in this complex enzyme system (Jacobson *et al.*, 1989; Zheng *et al.*, 1993). These results were followed by the identification of a similar set of genes (*isc*) elsewhere in the chromosome of *A. vinelandii* (Zheng *et al.*, 1998), and the subsequent demonstration that these genes were conserved from bacteria to humans, and had a critical role in the biogenesis of Fe-S clusters *in vivo* (Campos-Garcia and Soberon-Chavez, 2000; Hoff *et al.*, 2000; Lill and Kispal, 2000; Mansy *et al.*, 2002; Schwartz *et al.*, 2000; Seidler *et al.*, 2001; Skovran and Downs, 2000; Tachezy *et al.*, 2001; Takahashi and Nakamura, 1999; Voisine *et al.*, 2001). While the biochemistry of the gene products has been pursued in a number of organisms, research on the genetic locus as well as its regulation, and physiological role of the gene products has been most rapid in model organisms (Hoff *et al.*, 2000; Kambampati and Lauhon, 2000; Kambampati and Lauhon, 1999; Ollagnier-de-Choudens *et al.*, 2001; Schwartz *et al.*, 2001; Smith *et al.*, 2001; Tokumoto and Takahashi, 2001; Urbina *et al.*, 2001). The emerging characterization of the *suf* genes (recognized

now to be at least partially redundant with the *Isc* system) provides more evidence of the productivity that can be obtained by studying the relevant problem in a model organism, and combining these results with the input of data from work in other systems (Patzer and Hantke, 1999; Rangachari *et al.*, 2002). The rapid growth in this, and other, fields of metabolism can be largely attributed to the presence of comparative genomic technologies.

A slightly different example involves identification of a locus in the model organism, that has a homolog identified or studied for its role in a metabolic process(es) in a distinct organism. If, as is often the case in genetically tractable organisms, the lesion was identified by a phenotypic screen, the studies of homologs can provide a new perspective for the functional characterization that follows. The recent work on competence gene homologs in *E. coli* by Finkel *et al.* is a good illustration of this scenario. In this case, use of novel phenotypic analyses (*i.e.*, competitiveness in long-term stationary phase), led to the identification of genes required for the ability of *E. coli* to use DNA as a nutrient (Finkel and Kolter, 2001). Although not surprising in retrospect, the genes involved were homologs of genes in *Haemophilus influenzae* and *Neisseria gonorrhoeae* that had been ascribed a role in natural competence (Dougherty and Smith, 1999; Smith *et al.*, 1995). This finding facilitates progress in the understanding of two distinct, but similar metabolic processes. In the absence of comparative genomic technologies, each of the research groups would have a more difficult time identifying a function and physiological role of the gene(s) involved in their respective processes. This example also highlights the benefit that would be derived from knowing the physical identity of genetic loci that have been described in the literature. Such a correlation would allow researchers to take advantage of the physiology and phenotypes that have been described for mutants in multiple systems throughout the years.

While the above examples are directly impacted by the plethora of genome sequences and comparative genomic technologies, arguably the most important work in model organisms is the continuation of the efforts aimed at understanding basic metabolism that have been preformed for decades. This kind of methodical metabolic and molecular work will continue to define metabolic processes and paradigms, thus facilitating work in other systems. One should remember that a major factor in the speed at which genomic analyses of organisms progresses is the base of metabolic knowledge, and molecularly defined paradigms that have arisen as a result of decades of rigorous biochemistry, genetics, and molecular biology in the model organisms. Model organisms provide the logical forum to continue the molecular characterization of cellular processes, and uncover new metabolic paradigms. Without this work, the risk exists that analysis of sequence data will degenerate into no more than a means to catalog genes and proteins. A mechanism must remain in place to uncover new areas of research and functional paradigms. Genomic and sequencing technologies have not provided a magic bullet for understanding metabolism, they are simply one more tool in the arsenal available to the modern microbial

physiologist. The need for solid basic metabolic research in model organisms is unlikely to diminish in the foreseeable future.

Model Organisms Facilitate Genomic Analysis of Diverse Organisms

The use of model organisms goes beyond analysis of their own metabolism. Model organisms are designated as such because they offer technical ease, a property that can be useful in probing the metabolism of other organisms. The annotated genomes of model organisms provide functional information that is more difficult to access in less tractable systems. For instance, complementation of a mutant phenotype in genetically less tractable organisms by an *E. coli* clone of known function can provide insight about the function disrupted in the parent strain. Conversely, genetically defined organisms can be used to identify functional homologs from diverse organisms. Plasmid libraries of DNA from an organism of interest can be generated in a vector that can replicate in *E. coli*. When these plasmids are introduced to the appropriate mutant strain of *E. coli*, the plasmid(s) that complement the defect are candidates for carrying a functional homolog of the protein missing in the *E. coli* mutant (Bull *et al.*, 1994; Pascopella *et al.*, 1994). Use of heterologous systems (often but not always *E. coli*) has become prevalent in identifying genetic or biochemical functions from less tractable systems. The technologies available, in combination with comparative genomic capabilities have almost made *E. coli* a required lab reagent.

Metabolic properties of diverse organisms can be identified and studied by introducing the genetic material required for the relevant metabolism into model host organisms (Handelsman *et al.*, 1998; Rondon *et al.*, 1999; Weinstock *et al.*, 2000). This approach has been championed in the emerging fields of metagenomics (Rondon *et al.*, 2000) and metabolic engineering (Cameron and Chaplen, 1997). In the former, metabolic capabilities (*e.g.*, antibiotic production) can be identified even in the absence of a culturable parent organism. Appropriate host strains are identified based on the metabolic process of interest and other properties. Using this principle, metabolic capabilities can be "mined" from any environment by obtaining, and cloning, heterogenous DNA preparations. Following introduction of the resulting clones into the appropriate organism, a metabolic capability of interest can be identified as a "gained property" of the host organism. In the second, slightly different situation, organisms with properties of interest are used as a source of donor DNA. In this case, the desired metabolic property is often an ability to biosynthesize an antibiotic, or a given compound whose biosynthetic pathway is the target of engineering focus (Trauger and Walsh, 2000). The primary assumptions of these approaches are that, i) the host organism will provide "supporting metabolism" such that what is identified is the genetic information uniquely needed for the respective metabolic property, and ii) the genetic material for this process is located in a single region of the donor chromosome, such that it will be contained on a contiguous piece of DNA. These assumptions are more or less valid

depending on the situation, but the ease at which these kinds of approaches can now be applied, have attracted the attention of investigators in academia and industry. After the genetic material is identified from *in vivo* (or *in situ*) sources, it is useful in metabolic engineering efforts, where DNA encoding desired functions can be manipulated and/or mutagenized, to design and modify pathways for commercial gain (Aldor and Keasling, 2001; Aldor *et al.*, 2002; McDaniel *et al.*, 1999; Pfeifer *et al.*, 2001; Pfeifer and Khosla, 2001; Stachelhaus *et al.*, 1995).

In the above cases illustrating the potential for studies with heterologous metabolisms, the limitations of the system should be kept in mind. While these approaches offer a powerful starting point, the integrated metabolism of each organism is the result of years of selective pressures and the subtleties of regulation and efficiency are unlikely to be mimicked in heterologous systems. While manipulating these genomes and organisms to reach a goal or target a component, the benefit of understanding homogeneous systems should be kept in mind and pursued at a later point.

Functional Genomics - Or Classical Genetics?

At some level the goal of all microbial physiologists is to understand the function(s) of each protein and how they work together to result in the efficient physiology we associate with a living cell. The work of most investigators falls somewhere along the continuum of identifying a gene, to understanding its role and interactions *in vivo*. For a number of reasons, few labs strive to span the entire continuum. The more common scenario is when a genomic investigation identifies gene(s) that are connected (*i.e.*, by regulation, location, homology, etc.) to the area of research focus in a laboratory. In these situations, a putative function for the gene product often exists based on information that led to an interest in this gene. The function of the relevant gene is then pursued in the context of other research in the laboratory. Another powerful means to identify functionally connected components *in vivo* is by using genetic suppressor analysis. This approach will identify genes that are involved in a process of interest, however indirectly (Downs and Escalante-Semerena, 2000; Enos-Berlage and Downs, 1997; Enos-Berlage *et al.*, 1998; Gralnick and Downs, 2001; Petersen *et al.*, 1996). The difficulty in this approach is that genes that are identified by these mutant analyses often have no obvious connection to the process of interest. Thus the investigator may struggle to define the function of the relevant gene product and the explanation for its interaction with the initial process. So, while this approach has significant potential to provide a functional context for unknown genes *in vivo*, few investigators take advantage of it.

Efforts aimed at identifying the function of proteins on a global scale fall under the poorly defined rubric of "functional genomics". Often these approaches depend on a transcriptome, proteome, metabolome or the result of characterization by some other "omic" technique (Eymann *et al.*, 2002; Tao *et al.*, 1999; Tumbula and Whitman; Wen and Burne, 2002). Many times these efforts simply result in a catalog of genes with respect to a given condition or

genetic background. While these analyses provide valuable data, they are not a demonstration of function. One must realize that cellular function implies a known role *in vivo*. Understanding the function of a protein *in vivo* requires that two things be true. First, there must be a biochemical function for the protein that is demonstrable *in vitro*. Second, lack of this biochemical function must explain the phenotype that results in a cell lacking the relevant protein. Hence neither result alone, and certainly not a solely global analysis, will allow definitive annotation of a gene. A common "functional genomic" strategy being pursued in a number of organisms is to knock out all genes in turn, and analyze the resulting phenotype. While this sounds promising at first, the value of this approach is limited. Consider that in some organisms, notably *E. coli*/*S. enterica*, classical genetic approaches, scoring a variety of phenotypes, have been performed for more than 50 years. Without creatively screening phenotypes, how likely is this strategy to uncover genes that have not been identified? In the best case scenario, this approach may identify a few additional genes whose absence results in a clear growth phenotype. An immediate benefit could be derived from these approaches, if the data were interpreted in the context of past literature to assign a physical location to genetically defined loci in the literature (Dougherty and Downs, 2003; Frodyma and Downs, 1998; Roberts and Reeve, 1970; Sanderson and Roth, 1988). After decades of metabolic genetics and biochemistry in a number of organisms, our understanding of basic metabolism is good. Pushing the understanding of metabolism/physiology to the next level will require that creative approaches be used to address phenotypes caused by lack of additional cellular components. It is reasonable that many of the ORFs that remain completely uncharacterized are not required for a process to function, but rather for its optimization. Other genetic approaches must be considered to uncover the subtle and/or conditional defects associated with loss of these accessory or redundant proteins. Strategies that have been successful in identifying proteins with this type function protein include generating synthetic lethal mutants (or synthetic auxotrophs), scoring reduced not eliminated growth, and multi-copy suppression analyses (Gralnick and Downs, 2001; Petersen and Downs, 1996; Petersen *et al.*, 1996; Petersen and Downs, 1997; Trzebiatowski *et al.*, 1994). Other creative approaches wait to be pioneered.

Even at their best, the scenarios described above will identify a condition where the relevant gene product is needed, a far cry from knowing the *in vivo* biochemical function. The number of proteins whose only homologs are other undefined proteins with a similar motif, suggests we do not have an understanding of all functional classes of proteins. It is possible, and even likely, that these protein families define new functional and biochemical paradigms that have not been characterized. The question becomes how to go past cataloging by sequence similarity and expression studies to identify the biochemical function? This question is at the crux of an ability to extend our understanding of metabolism, and there is no easy answer. Nor is there a computer program with the power to predict function from unique primary sequence. It has been suggested that by determining the crystal structure of these

uncharacterized proteins, a putative function will be clear. While this structural genomic approach is being pursued in a number of research groups, it is unclear that it has been successful in identifying a function of a completely novel protein (Eisenstein *et al.*, 2000; Volz, 1999; Xu *et al.*, 1999; Zarembinski *et al.*, 1998). It is more likely that work toward functional elucidation will proceed by meticulous “discovery” science that is facilitated by metabolic genetics and biochemistry. Progress in this work cannot be outlined, nor forced, because the outcome is not predictable. These studies must be pursued with a creative mind and the conviction that new paradigms are yet to be discovered. Investigators successful in this research will use molecular, biochemical, genetic and genomic technologies, and follow their scientific intuition. This strategy has resulted in landmark discoveries in the past, and genomic technology has yet to eliminate the need for this kind of unbiased discovery based research.

Data Management and Dissemination

With genomic technologies have come unprecedented volumes of data that must be cataloged, mined and analyzed. This is a major challenge for computational scientists, and significant efforts are being made to ensure data management keeps pace with progress of genomic technologies (Covert *et al.*, 2001; Edwards *et al.*, 2002; ;Edwards *et al.*, 2001; Edwards *et al.*, 2002; Krauthammer *et al.*, 2002; Mendes, 2002; Palsson, 2002; Papin *et al.*, 2002; Wagner, 2001). While the challenges of designing software to handle, screen and analyze these data are recognized, there are additional data management and dissemination needs required for research in bacterial metabolism to progress efficiently. These challenges include the need to: i) make data broadly available, ii) rigorously and rapidly update genomic annotations, and iii) define common nomenclature rules. The field of metabolism and physiology is characterized by its integrative nature. Progress in this field, more than most, is dependent on the access to knowledge of numerous pathways/processes. Because information that must be considered in metabolic studies is becoming so diverse and widespread, it is often not identified by current researchers and this slows progress and increases redundancy of effort. Much of the potential for efficient progress promised by global techniques will be lost if the scientific community cannot simply determine what data are there to support one or another conclusion.

Correct and current annotation of sequenced genomes is critical for metabolic studies. As mentioned above, significant work on unknown genes, and new genes in diverse organisms is dictated by sequence similarity to annotated genes in model organisms. If investigators are allowed to describe the function of a gene based solely on similarity to an annotated gene in a different organism, and the respective annotation is incorrect or not definitive, the literature can become compromised in a way that is hard to reverse. It is imperative that biologists be involved in updating the annotations and that the standard for annotation be clearly stated in each case, such that appropriate, defensible, conclusions can be made when

sequence similarity is detected.

Post-genomic nomenclature is also a concern for the efficiency of metabolic studies (Fields and Johnston, 2002). Genes have traditionally been named based on phenotypic analysis and this has led to problems when the biochemical function of the gene product is determined (Frodyma and Downs, 1998; Skovran and Downs, 2003; Trzebiatowski *et al.*, 1994). A better solution is the current trend to name genes based on chromosomal location (*i.e.*, YXX, or STM) until a biochemical function can be attributed to the gene product (K. Sanderson and M. Berlyn, personal communication). This is not yet a perfect solution, since, in *E. coli*, both a “b” designation, and the “yxx” designation exist and are used. Unfortunately, the literature has been permeated by cases where multiple names for the same gene are used. Not only is this a problem that is tedious and time consuming to fix, it is unclear whose domain such an effort should fall in. An extension of this issue is the realization that a large amount of useful phenotypic information is present in the literature from the time when loci were genetically, but not physically defined. If a genetic/physical correlation of these genes were generated, it would allow data from the past to be better incorporated in the context of current work, which is preformed with the mindset of physical location. Unfortunately, in both of the above cases, work to clarify the literature, and eliminate redundancies is unlikely to be preformed on more than a case by case basis unless a major emphasis is put on solving these data management challenges.

In addition to the rather mundane cases described above, there is a growing need for new computational technology to integrate information present in the literature. For metabolic research to move forward productively researchers must be able to consider their results in the context of what is known. We are now in an exciting time where data integration is possible and global thinking is encouraged. Some researchers can be well versed in the connections that exist in a small area of metabolism, but as the understanding of metabolic connections and integration expands, it becomes less feasible for this information to be stored by single investigators.

From the perspective of bacterial metabolism, the challenge facing the computational scientists is to design software that can, i) integrate the information in the literature on individual pathways and processes to present an accessible picture of the metabolic connections that exist *in vivo*, and ii) scan literature for words or phrases that may be buried within a manuscript of a different focus. In the former case, storage of known connections in a form that can be accessed, modeled, and expanded is needed. Some of this work is beginning, and several databases built with this goal in mind exist (Goesmann *et al.*, 2002; Karp, 2001; Karp *et al.*, 2002; Karp *et al.*, 2002). As our progress in understanding metabolic integration continues, there should be some mechanism to incorporate these basic research results into the metabolic databases. Display of this information in a useful way will require some creativity. Both direct biochemical connections, and more indirect connections, which can also affect function of a pathway/process, need to be presented. Importantly, these databases need a clear description of which connections/

activities are documented by experimental data and which are simply hypothesized.

The second case reflects a need for investigators to find references to a particular metabolic process from the literature when the relevant statement may be a small piece of a manuscript. Classical approaches to metabolic characterization often result in valuable observations, which appear peripheral to the original focus of the project. The significance of these peripheral observations may not be apparent to the authors of the original study. Yet, to investigators focusing on a different area of metabolism, these observations may provide critical insight. In such a scenario, the "side" observation might never be accessed by the relevant researchers, since as a minor statement in the manuscript, it would be invisible to standard literature searches. An ability to search the literature for words and phrases that indicate a metabolic connection would facilitate the integrative thinking that investigators in metabolic studies need. Progress in metabolic studies depends on astute researchers recognizing phenotypes and observations that suggest a key connection or explain a metabolic behavior. The difficulty in the field has been communicating these observations in a way that the relevant person(s) has access to it in their thinking. This area cries out for collaboration and communication between experts in a number of disciplines, and identifies an area that could have an enormous impact on the progress of our understanding of bacterial metabolism. When this concept becomes a reality, perhaps the scientific community as a whole will be more receptive to the value of describing phenomena for which a biochemical explanation is not yet readily available.

Conclusions

These are exciting times for the field of bacterial metabolism and physiology. It has long been recognized that the cell was comprised of many integrated metabolic pathways and processes. As our knowledge of individual components has increased, we have reached the point where it is no longer enough to consider a single pathway (or process) without regard its integration with the other cellular components. As global technologies and data management strategies continue to be developed, the capacity to present and catalog the integration of multiple pathways and processes will reach a level not possible for the human brain to retain. These capabilities will push our understanding of bacterial metabolism to a higher level of complexity.

Ironically, what seems to draw researchers to genomic analyses, *i.e.*, the volume of data reflecting activity in the whole cell, is similar to the characteristics that often drive investigators from the more classical approaches to physiology and metabolism. The methodical "discovery" science of classical metabolic genetics and biochemistry is often characterized by slow paced, weaving progress with numerous wrong turns, required to generate and discard models that can explain complex phenotypes in the context of the cellular biochemistry. The field of bacterial metabolism is characterized by a self-imposed mandate to consider metabolic processes in the context of cellular physiology, rather than in isolation. The difficulty these

researchers face is the volume of data, and knowledge of diverse metabolic pathways they must be able to draw from to generate an inclusive model. Computational science has the potential to ease this difficulty in the future and facilitate the integration of data generated from multidisciplinary approaches to metabolism.

Genomic technologies and data analysis techniques are powerful tools to probe bacterial metabolism but they have yet to replace the curiosity and creativity of the human mind in making progress. These technologies have allowed the rapid cataloging of sequence similarities and differences, common regulatory themes and protein stability. The challenge in the field of physiology and metabolism in the future will be to take advantage of these technologies without losing an open mind and the drive to pursue the exceptions, or the pieces that do not fit existing paradigms. Thus, in addition to genomic technologies, creativity and persistence will be required if we are to take our knowledge of cellular processes and understanding of metabolic integration to the next level by continuing to define biological paradigms.

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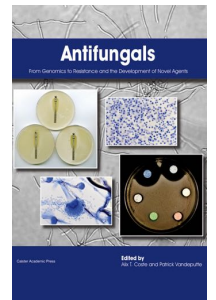
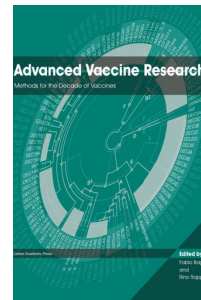
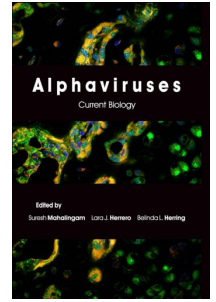
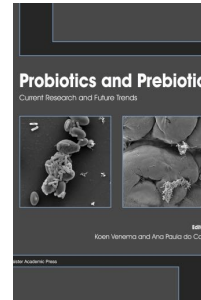
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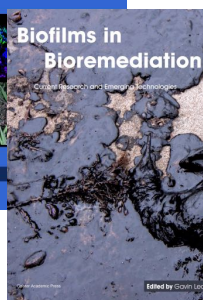
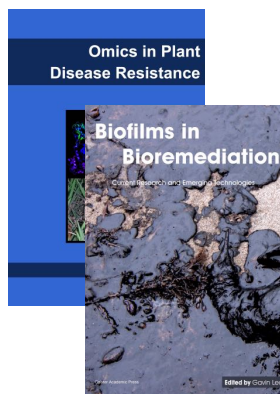
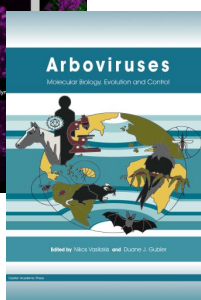
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