

## Integrative microbiology – the third Golden Age

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### 1. Some personal vignettes

I am taking advantage of advancing age to unravel myself from the narrow confines of my field of research and present a broad personal view of my science, microbiology. Before commenting on my views of present-day microbiology, let me disclose some of the personal experiences that may have had a defining role in my scientific development. I mention them with circumspection because the relationship between early events and later actions may be misleading. Still, sharing aspects of what I experienced in my early days may help to set the stage for my present-day musings. Those eager to get to the message should skip this section.

I was born in Italy, where my parents, who were Polish Jews, had lived for many years. In 1940, we left for Ecuador as refugees and I was twelve years old. My earliest yearnings for studying bacteria began at the age of 14, when I read Paul De Kruif's *Microbe Hunters*. This book is an exaltation of the early pioneers of microbiology written pretty much for adolescents. Not only did I read the book once, I re-read and memorized parts of it. Each one of the people described seemed as fearless, as reckless as any great adventurer who faced insurmountable odds. I remember my mental anguish about the injustices committed to such noble sounding figures as Spallanzani, Pasteur, Ehrlich, and Bruce. I became committed to go down the same path and brave the slings and arrows that undoubtedly would befall me as I would be blazing the trail towards the cure of infectious diseases. Quite a few other microbiologists relate the same experience, about how this book influenced their career choice.

I spent my last four years as a teenager working part-time in a laboratory of an industrial company in Quito, Ecuador. The people in charge of this laboratory did not mollycoddle anyone and in my first summer there at the age of 16 I was put to work doing menial tasks. I first worked in the media kitchen where we had to make all

the nutrients for bacteriological cultures from scratch, grinding up meat and making various broths and agars. A break from this routine came when a cow from the stable that the company maintained for various purposes had died. In the frugal ways for which the management was famous, nothing went to waste. I was given the heart and told to turn it into powder for the preparation of the reagent used in the classical (and long superseded) Wassermann test for syphilis. This test depended on the reactivity of non-specific antibodies in patients' sera with the phospholipid cardiolipin, which is found in all tissue. Heart muscle was a favorite source of this reagent. Making the Wassermann reagent was not a small matter, it turned out. First, I had to grind up the heart and dry it into pungent, dark brown, hard shapeless lumps. I was given a porcelain mortar and pestle to convert the stuff into a fine powder. This became a challenge, as each lump had to be pounded and crushed one by one. A fragment larger than a pinhead was unacceptable. I must have spent a whole week doing this, counting every hour and minute.

Later on, I graduated to more sophisticated tasks, each of which presented a particular challenge. There was little in the way of instruction and I had to figure out many techniques unaided. I remember that once my superior, an Italian by the name of Aldo Muggia, unceremoniously dumped on my bench yet another piece of equipment. It was a freezing microtome, the gadget used for making the frozen sections that pathologists use for rapid diagnosis of biopsy specimens. In this case, it was the handiest way of getting started making tissue sections (paraffin sections came eventually). My boss said something to the effect of "Figure out how to make sections," and left. I don't know of anyone else who has tried this without being shown how to do it, but let me say that the challenge is not inconsiderable. It took me several weeks before learning how to make passable tissue sections. My boss also had an instant cure for squeamishness. He sent

me to the hospital for contagious diseases and told me to obtain fecal specimens *directly* from the patients and to culture typhoid bacilli from them. I used throat swabs for the anatomically opposite end they were intended for. The ward was the closest to a medieval lazaretto, but a solution of phenol was available to the staff. I burned my hands by using it repeatedly and generously.

In 1950, I went to the United States for graduate work, in good measure because the opportunities for becoming a scientist in Ecuador were limited at the time. I have a warm spot in my heart for that country because it not only took us in and only allowed us to survive, but also generously provided me with an appropriate high-school education. I began my graduate work at the University of Kansas, then went to the University of Pennsylvania, where I did a thesis on the nuclear cytology of the alga *Chlamydomonas*. No sooner did I finish it that I was drafted into the Army. Luckily I was sent to a research lab where I was to work under the supervision of an eminent rickettsiologist, Joseph Smadel. In those days, it was not entirely clear whether rickettsiae (and chlamydia, for that matter) were bacteria or viruses. There were even whispers that they might constitute an intermediate group. Smadel was clearly tired of this kind of talk and proposed that I solve the problem once and for all by looking at rickettsiae in living infected cells and following individual organisms. Would they divide by binary fission? An obstacle that suggested itself was to distinguish rickettsiae from mitochondria under the phase contrast microscope. This turned out not to be a serious problem because the rickettsiae had a rod shape typical of many bacteria and had greater contrast than the mitochondria. Armed with this knowledge, one happy day I was able to follow three individual rickettsiae until each indeed divided into two, some 6–8 hours later (Schaechter *et al* 1957). One does not need reminding today that rickettsiae are bacteria but I tell the story for a reason. In recent years, the genome of mitochondria has turned out to have the greatest kinship with that of rickettsiae (Andersson *et al* 1998). In our work we had to distinguish between an organism and its related organelle, a pleasing thought. Obviously, we did not realize that we were witnessing a family reunion.

My experience with microbial cytology influenced my postdoctoral work with Ole Maaløe in Copenhagen. When I got there, Ole was changing his interests from bacteriophages to bacteria and work in his laboratory led in time to the creation of the influential Danish school of bacterial physiology (Maaløe and Kjeldgaard 1966; Ingraham *et al* 1983; Neidhardt *et al* 1990; Cooper 1993). On my very first day there, I caught on that science there was done differently from than what I was used to in the United States. I came to work at what I thought was a

reasonable hour, 9 am. There was nobody there, and people drifted in gradually over the next two hours. The usual routine was for the two post-docs, Niels Ole Kjeldgaard and myself, to first inoculate a fresh culture and then spend several hours discussing with the boss the results from the previous day and planning for the experiments to be done that afternoon. We were studying growth physiology of *Salmonella typhimurium* and uncovered the linear relationship between the growth rate and the RNA and DNA content of the cells. Doing innumerable viable counts and looking at the bacteria under the microscope was a crucial aspect of this work. Later on, we measured the content of ribosomes in cells growing at different rates and found, to our delight, that there was a simple relationship here too (Ecker and Schaechter 1963). The faster the growth rate, the more ribosomes there are per cell mass. In other words, the rate of protein synthesis turned out to be a linear function of the concentration of ribosomes (Schaechter *et al* 1958; Kjeldgaard *et al* 1958). Ribosomes, then, operate at close to a single unit of efficiency, regardless of whether they find themselves in a small, slow-growing cell, or a large, fast-growing one. Another way of saying it is that the rate of polymerization of proteins (the chain growth rate) is nearly constant as long as cells are growing. This research led us to conclude that bacteria can exist in a continuum of physiological states dictated by the growth rate alone. Thus, we could establish some order in the previous chaotic knowledge that cells of a given bacterial species can vary in shape and composition. As long as bacteria are growing unhampered, i.e. are undergoing balanced growth, their global phenotype can be predicted. This helped establish a reproducible baseline for studies involving bacterial growth. The general conclusion was that bacteria in different media are different and obey the maxim of the Spanish philosopher Ortega y Gasset: "I am I and my circumstance" (*Yo soy yo y mi circunstancia*).

This way of thinking helped others to do further experimentation that revealed much about the mechanisms that control gene expression in bacteria. How is the synthesis of the RNA of the ribosomes regulated? What about the synthesis of ribosomal proteins? What does this have to do with how gene expression is regulated? This approach was extended by Helmstetter and Cooper (1968) to the regulation of chromosome replication, leading to the realization that the process is controlled at the level of initiation and that, over a range of growth rates, the speed of DNA polymerization is also nearly constant (Maaløe and Kjeldgaard 1966).

At the Tufts University Medical School in Boston, I went on to study other aspects of bacterial physiology, including aspects of ribosome transactions, fatty acid metabolism, and the role of the cell membrane in

chromosome segregation. Like any older scientist, I rue that some early leads from my laboratory have not been followed up. I will mention one (Green and Schaechter 1972). My student Betsy Green determined that if bacteria labelled in their lipids were allowed to grow in unlabelled medium (a “chase”), some of the label did not disperse totally among the progeny cells. After eight generations, one in about two hundreds cells retained the label, suggesting that the cell membrane is partitioned in conserved “rafts” that are about 0.5% of the total structure.

Towards the end of my active research years at the Tufts Medical School, my colleagues and I spent considerable time trying to puzzle out some of the transactions of the bacterial chromosome. We found that the origin of chromosome replication in *Escherichia coli* binds selectively to membrane preparations, but only when it is hemimethylated, i.e. is newly replicated (Ogden *et al* 1988). This finding, together with those from Nancy Kleckner’s lab at Harvard (Campbell and Kleckner 1990), suggests that binding to the membrane impedes premature initiations that would be deleterious to the cell. Alas, probing this kind of question has not proven to be easy and our knowledge of the microbial equivalent of mitosis remains unsatisfactory.

Throughout my career, my outlook has remained cell-oriented and tending towards the whole bacterium. Somehow, I never seemed to lose sight that bacteria are living entities. I have not been particularly drawn to examining molecular mechanisms in depth, although I certainly admire the power and intellectual satisfaction of this approach. With age, I am drawn more and more to learn about the breadth of the microbial world. There seems to be no end of surprises in microbial ecology, with organisms being found daily in unusual and unexpected habitats. I especially derive pleasure from the splendid variety of interactions between microorganisms. Symbioses between prokaryotes as well as with eukaryotes consistently represent elegant solutions to the problems of coping in varying environments (Margulis 1998). My interest in the microbial world that is outside the laboratory is expressed in a hobby I have developed, the study of wild mushrooms (Schaechter 1997).

## 2. The first Golden Age

I would like to place some of my views of the current state of microbiology in a historical context. This science has enjoyed splendid chapters in its history. It made a grandiose entry at the end of the 19th century, thanks to its modern progenitors, Pasteur, Koch, and their students. From the beginning, microbiological research and its applications have had affected health, nutrition, and the

environment and, consequently, have had a pervasive effect on society. Remarkably, the discovery of the microbial etiology of major infectious diseases took place in the first twenty years and led in turn to accurate diagnoses and attempts at prevention and cure. Several vaccines used today stem from those developed by early microbiologists. Equally important, early research made it possible to understand the cycles of matter in nature, as well as providing a rational basis for food production and preservation. This was the first Golden Age of Microbiology.

During the first few decades of the 20th century, microbiology became split into discrete, often divergent branches. The unity that Pasteur originally brought to the discipline by using similar approaches to medical and environmental microbiology was replaced by specialized identities. During this period, medical microbiologists and immunologists were mainly doing research on the organismic level, trying to integrate the activities of host and parasite. Environmental microbiologists, on the other hand, were focused on chemical processes. Thanks to the pioneering work of the Russian Sergei Winogradsky and the Dutch Martinus Beijerinck and Albert Kluyver, environmental microbiology became a branch of comparative biochemistry. Its emphasis was on the unity of the biochemical processes in all living organisms. A story from my experience illustrates the rift between these two branches of microbiology even at a later date. In the 1950’s, I was fortunate to attend the fabled microbiology course at Pacific Grove, California, taught by an eminent exponent of the Delft school, Cornelis Van Niel. I noticed that the incubators were set at 5° intervals (30°, 35°, 40°, and so forth). I asked Dr Van Niel why he did not have one set at 37°. He snorted: “That’s medical!”

Broadly speaking, microbiology in this period had not yet become a cellular science. The rest of biology had at least the benefit of extensive cytological investigations, although cell fractionation techniques and electron microscopy had still to exert their full force. Experimental biology at the time was nearly overshadowed by biochemists and their superlative achievements, the elucidation of central metabolism. However, this did little towards understanding bacteria as cells and their endowment of a complex organization. Biochemically-oriented researchers treated bacteria as particles that, inconveniently, one had to grind up with sand or blow up by decompression. The main bacterial cell components – cell wall, membrane, cytoplasm, and nucleoid – were only fuzzily envisioned and became the subject of intense and sometimes passionate controversy among the few who seemed to care. The work of only a few insightful researchers, including later on that of the Canadian workers Carl Robinow and Robert Murray, has held firmly over the years. Outside this fledgling field of bacterial cytology (as it was then

called), many viewed bacteria, and, God-knows, viruses, as somewhat peculiar entities off the beaten path. It was not even agreed whether bacteria were *cells*, leave alone whether they possessed genes or not. The attitude of these times is illustrated by the relative indifference that greeted the stunning discovery of genetic transformation by Fred Griffith in 1928.

### 3. The second Golden Age

The second Golden Age of microbiology materialized in the 1940's with the birth of molecular genetics. This era was heralded in 1943, by the classic experiment of Salvador Luria, a microbiologist and Max Delbrück, a physicist, using a bacterial system to address a fundamental question in evolutionary biology, viz. whether mutations are spontaneous or induced by the selecting environment. In dramatic fashion, this experiment pointed out the usefulness of bacteriophages and bacteria as model systems for the study of some of the most important questions in biology. Soon thereafter, in 1946, Joshua Lederberg discovered conjugation in bacteria. The importance of this experiment transcended genetics because it led to the appreciation that if bacteria can mate, they must be cells.

These celebrated achievements were followed by other path-breaking discoveries in the early 1960's on fundamental aspects of biological processes such as the structure of DNA, the existence and importance of plasmids, the regulation of gene expression, and the elucidation of the genetic code. Now, to be at the forefront of biology and at its cutting edge, one had to work with microbial systems. Coincidentally, antibiotics came into widespread use and one of the classical arms of microbiology, the study of pathogens, waned in importance. In fact, some declared that the infectious diseases were about to be conquered and, by extension, that medical microbiology would become a relic. Like the premature announcement of Mark Twain's death, this one proved to be grossly exaggerated. Nevertheless, for a period between 1950 and 1970, microbiology became almost synonymous with molecular biology.

In time, the lessons learned from microbes were found to be applicable to the study of eukaryotic cells. For those that can be manipulated with nearly the same ease as bacteria, e.g. yeast, the transition was a direct one. Other systems that lack the ease of manipulation and the speed of growth of bacteria, such as animal and plant cells in culture, benefited proportionally even more from DNA cloning and sequencing. Thus, the playing field became more even. This led to a substantial flight of researchers from bacteria and phages to eukaryotic cells, abetted in the early 1970's by Nixon's declaration of "War on Can-

cer." As funding for basic microbiology became increasingly difficult to obtain in the United States and elsewhere, many aspects of this science became neglected and were left to a few stalwarts. For example, the once thriving area of bacterial and plasmid DNA replication is now in the hands of a few investigators.

### 4. The third Golden Age

The fortunes of microbiology have now changed again and there are signs that this science has entered its third Golden Age. The reasons for this assertion can be seen in the following advances.

#### 4.1 *Microbial ecology*

Once a specialized branch of microbiology, microbial ecology is now occupying center stage. Thanks to cloning techniques and PCR, it has become possible to break out of the confines of model organisms and to carry out meaningful studies on almost any microorganism. This includes microbial species that cannot yet be cultured in the laboratory, estimated to comprise over 99% of the total (e.g. Eilers *et al* 2000). It is now possible to approach questions such as who lives where, who does what, and who is phylogenetically related to whom. The consequence has been a huge expansion of the microbial world, even embracing whole new kingdoms such as the Archaea. Microorganisms have been found in unexpected niches, such as thermal ocean vents and fissures in deep rocks (e.g. Taylor 1999). Such lithotrophic organisms support the existence of biomes that are not ultimately dependent on the sun or atmospheric oxygen, thus expanding the scope of microbial ecology and our understanding of the role of microbes in the cycles of matter in nature (Postgate 1994).

There is also an increased appreciation that microbes in nature tend to live in communities, some with their own kin, others together with other species. Such realizations have made it imperative to extend the study of microorganisms from the laboratory to where they live in nature. In addition, pathogenic microbes are increasingly being investigated in their natural habitat. Researchers such as Stanley Falkow and John Mekalanos and their students have devised ingenious genetic manipulations to identify those genes that are selectively expressed in the host. This ecological approach has led to great strides in the identification of novel mechanisms. An example is the so-called type III secretion, a device used by bacteria to "inject" proteins directly into the host cells and which is turned on by contact with host cells (Buttner and Bonas 2002). This implausible-sounding mechanism is

shared by a surprisingly large number of pathogens of vertebrates, insects, worms, and plants. Perhaps the gamut of microbe-host interactions is not as extensive as it may have been envisaged in the past.

#### 4.2 Evolution

In part because of their size, the genomes of many more prokaryotes than of eukaryotes have been sequenced. The number of completed sequences includes not only representatives of major groups but also multiple strains of a given species. Not surprisingly, this richness of data has spawned a whole industry intended to “mine” information of practical use. Genomic information has been successfully used to trace the path of evolution. One surprise has been the demonstration of the extent and impact of lateral gene transfer (Sonea and Mathieu 2002). Genomic islands, sets of genes a dozen or more in number, appear in an unrelated species and endow them with new functions such as a virulence or a novel metabolic capacity. Thus, bacteria break out of the confines of sexual recombination by a set of more promiscuous mechanisms that allow gene transfer between unrelated species. The extent and popularity of lateral gene transfer is causing us to rethink the concept that evolution can be traced strictly along separated branches of a tree. Instead, the branches of the tree are laterally connected to one another. Thus, the ancestry of an organism is not simply monophyletic but is the result of multiple interactions between distinct genomes. There are hints that such interactions can also jump across the prokaryotic-eukaryotic divide. Incidentally, all this plays havoc with the concept of species in microbiology.

#### 4.3 Microbial cell biology

One recalls with amusement the old dictum that bacteria are just bags of enzymes. Within the small confines of one cubic  $\mu$  or so, there is a sophisticated and unexpected compartmentalization. Cellular components act in dynamic fashion and a surprising molecular choreography has been unveiled with fluorescence microscopy and other techniques. Some macromolecules localize to specific sites, others move around the cell interior along established paths. In nearly all prokaryotes, cell division depends on the formation at mid-cell of a constricting ring that consists of the tubulin-like protein FtsZ (Osteryoung 2001). Other proteins, called Min, move from one pole of a rod-shaped bacterium to the other, helping locate the division site. Rod-shaped bacteria also have an actin equivalent (Jones *et al* 2002). At least some of the cytoskeletal proteins, it turns out, were prokaryotic inventions that long preceded emergence in eukaryotes.

#### 4.4 Eukaryotic cell biology

Pathogenic agents make extensive uses of the machinery of their host cells, such as cell surface receptors, cell signaling pathways, or the cytoskeleton. An example is the suborning of host cell actin by *E. coli* to promote binding to intestinal cells or by *Shigella* and other bacteria to ensure their passage from one host cell to another. These phenomena help us understand the workings of eukaryotic cells. Nowadays, pathogenic microbiologists are being invited with increasing frequency to deliver papers at cell biology meetings. As in the old days, when the proper study of bacteria was via bacteriophages, it seems increasingly evident that studying bacteria-host interactions opens the door to many aspects of eukaryotic cell biology.

#### 4.5 Some consequences

These conceptual advances have practical uses that touch on many societal concerns. The most evident now is the threat of bioterrorism, which calls for improved ways of detecting pathogenic agents, preventing their activities and spread, and finding new ways to treat affected humans, animals, and plants. The search continues for antimicrobial drugs that will not elicit a rapid onset of microbial resistance, as well as for improved and new vaccines. Let us hope that such developments will help humanity as a whole. These concerns place a heavy ethical burden on microbiologists of all countries. There is no precise line to be drawn at present between what work is morally justifiable and what is malevolent in intent. My hope is that workers in the field will be able to make this distinction and act accordingly for the common good.

Microbial activities are having an increasing role in industrial production of pharmaceuticals and other compounds. There has already been considerable success in mining the genomes of cultivated and uncultivated microorganisms for enzymes with desirable physical and chemical properties. This approach has already brought to market several hydrolytic enzymes with enhanced thermal stability and targeted substrate specificities. The future looms bright in other areas as well. For example, fermentation processes have the potential to decrease our reliance on fossil fuels. An infusion of funds in these areas will almost certainly lead to significant advances, but it seems prudent to be mindful that practical success ultimately depends on the health of its scientific foundation. Let us hope that decision makers are aware that the well being of our planet and its passengers is increasingly dependent on the activities of microorganisms and therefore on the development of microbiology.

### 5. Integrative microbiology

There is more. Microbiology is becoming more unified. Microbiologists speak ever more a common language.

Nowadays, a marine microbiologist can easily talk to one studying human pathogens and a food microbiologist can converse effortlessly with one studying evolution. Pathogenic processes have become cases in microbial ecology, while microbes in the environment reveal evolution in the making. Those studying the microbial world share not just a genomic database but, more profoundly, the realization that microorganisms make use of similar capabilities for diverse purposes. Common molecular mechanisms of adhesion to surfaces, quorum sensing, signal transduction, constructing communities, or injecting proteins directly into host cells are found in dissimilar organisms that represent a broad spectrum of microbial life. As a result, the subdisciplines of microbiology are no longer isolated fields of study. Microbiology has become an integrative science, one that thrives to combine and coordinate diverse elements into a biological whole. This is the new culture of microbiology.

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