Prokaryotology

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PROKARYOTOLOGY
A COHERENT VIEW

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FOREWORD

This volume stresses the major differences between prokaryotes and eukaryotes, differences which are both profound and astonishing. They show that the general strategy of life in prokaryotes differs strikingly from that in eukaryotes despite the fact that many of the fundamental biochemical reactions are very similar in the two groups. However, too few scientists have as yet come to realize this. Our understanding of prokaryotic biology has progressed considerably with the in-depth studies of more than a thousand different types. We have learned to cultivate many of them, assess some of their properties and, for a small number, their role in nature. This vast and reliable new knowledge tells us that prokaryotes are ancient but not primitive, highly specialized and standardized cells which show an amazing capacity and tendency to collaborate among themselves. Most of them live in close association with each other in mutualistic, mixed groups (the metaphor of community is apt) practicing an effective division of labor. When advantageous or necessary, these communities can easily modify the mixture of their cells, replacing some strains with others, drawn from the prokaryotes'giant reserve of differently specialized cells. This allows them to react and adjust quickly and opportunistically to the changes around them. Moreover, in contrast with eukaryotes, individual prokaryotic cells are able to function as two-way communication stations, exchanging bits of hereditary information (DNA) which can be

easily and frequently transmitted horizontally between different strains. The exchange of prokaryotic cells between mixed communities, added to the constant and opportune horizontal transfer of genes, constitutes an original and efficient adaptation system which endows the prokaryotic world with the ability to perform very complex functions and solve problems. There are no species among prokaryotes. Rather, together they form one global, extremely diversified, yet functionally unified peculiar being.

It is from prokaryotic stock that the eukaryotes most probably originated, i.e. from the permanent association of a few complementary strains of the already rich and varied prokaryotic entity. This association gave birth to a completely new type of cell. Much later, other prokaryotes also joined some more evolved eukaryotes in successful symbioses. Thus, in parallel with their own evolution, the prokaryotes have been extensively involved in eukaryotes' evolution, contributing genetic information on a much larger scale than previously thought.

We postulate that the prokaryotic world behaves as a global system or superorganism of elements capable of solidarity. It has continually improved the life-supporting capacity of our biosphere and constitutes the main positive ecological factor on our planet. Its general way of functioning may be compared to the one practiced in a technically advanced economy. It is based on the ability to use, for its own needs, available nutrients and produce a large variety of products (metabolites) which can be exchanged for the temporary benefit of the different members of its population. In addition, the prokaryotic world practices among its constituents a permanent competition which selects locally favorable changes and eventually eliminates the less productive elements or associations with resulting increased efficiency and economy. This new vision of the prokaryotic world comes as a coherent counterproposition to the generally accepted idea that many thousand bacterial "species" live as independent infighting cells in a global chaotic mixture. In order to make our thesis

more convincing, we have presented all these aspects from several different angles, each, hopefully, adding some new light to the subject. This procedure did, however, give rise to some unavoidable repetitition.

In the 1970's, a new category of prokaryotes, the Archaebacteria (Archaea) was recognized and described. Many of these types have been studied since then, and it is evident that some of their bio-energetic and macromolecular characteristics are more closely related to those of eukaryotes than to those of Eubacteria. In fact, some biologists consider Archaebacteria sufficiently different from Eubacteria to support the suggestion that all living beings be divided into three kingdoms or domains: Eukarya, Bacteria and Archaea. Metabolic and macromolecular differences notwithstanding, we wish to stress the fact that Eubacteria and Archaebacteria share fundamental ways of life based on interdependence and solidarity. They include life in communities with efficient and adaptable division of labor and also active horizontal exchanges of genes among their strains involving the same general mechanisms that were originally observed and described in Eubacteria. To this may be added the fact that after a long evolution, likely from a common progenote ancestor, the size, shape and general organization of Archaeabacteria cells have retained essential similarities. For all these reasons we insist here mostly on important and general differences between eukaryote and prokaryote (Eubacteria and Archaeabacteria) patterns and strategies of life, differences we consider are not sufficiently emphasized and studied in Biology and Microbiology. Our purpose therefore is not to question the well established fact that diversity does exist among the members of the prokaryotic world. Rather, we wish to recall and discuss some essential biological differences between the prokaryotic and the eukaryotic worlds and their respective evolution. We consider these differences as essential elements on which a coherent study of prokaryotology can be founded and built. Understanding of the entire field of biology would benefit from this.

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UNEVEN DEVELOPMENT
OF THE SCIENCE OF BACTERIOLOGY

An impressive amount of knowledge has been collected over the years concerning the structure, metabolism, bio-energetics and genetics of bacteria (prokaryotes), but their real nature, in particular their entirely original life strategy and evolution, continue to be misunderstood or remain unknown (Mathieu and Sonea, 1995,1996a,b). Outdated and, unfortunately, often confusing concepts about bacteria can still be found in some biological publications and textbooks, and, regrettably, they may be transmitted at different educational levels. This causes a deformed and unbalanced view of whole chapters of biology instead of bringing clarity and arousing interest in the exciting world of the first living beings to settle on the planet Earth, the inventors of so many of the fundamental biochemical reactions essential to themselves and, later, to all eukaryotes. Ironically, in the teaching of evolution on Earth, the role of bacteria is vastly understated (Zook, 1995). Also, their essential and momentous participation at all echelons of the biosphere is often relegated to complementary readings, or superficially covered in the classroom.

As a consequence, despite continued scientific advances, the status of microbiology and of bacteriology in particular, as academic disciplines, has been eroded in the last decades, so that

microbiology was no longer included as one of the major biosciences in the 1995 survey of US graduate programs (Pelczar, 1996). Failure to give bacteria their rightful place and importance in biology has not encouraged young scientists to enter microbiology programs and several of the respective university departments have experienced restrictions from the funding agencies. As a reaction, some have changed their official name for more attractive, fashionable ones, and bacteriology faces the risk of splintering into several apparently unrelated subspecialties. Moreover, long-term consequences of neglecting bacteriology in favour of premature specialization in molecular biology are difficult to assess precisely but they may lead to a serious unbalance in the training formation of undergraduate students of biology and of health sciences.

1. The importance of pathogenic bacteria in the early developments of bacteriology

The peculiar way in which humanity has discovered bacteria and has gradually learned more about them is unique and it may explain in part why a correct vision of the bacterial world still lags behind the explosive accumulation of new knowledge. Nobody had seen prokaryotes before Leeuwenhoek, a little over three centuries ago. Following his description of "animalcules" observed through his microscope, the curiosity of a few scientists was aroused and some made additional observations but their significance could not be clearly established. These early contributions remained without meaningful consequences. Later, in the second part of the nineteenth century, Pasteur, Koch, and others proved that many human and animal infectious diseases are caused by bacteria usually identifiable in the laboratory, and by one specific kind for each type of infection. At the beginning of the twentieth century most of these pathogenic bacteria had been grown and studied with the help of increasingly standardized laboratory techniques. As a consequence, rational and

efficient public health measures could be devised and applied. Shortly thereafter, the uses of immunization and serotherapy were successfully tested and implemented. Specific drugs against pathogenic bacteria were sought and eventually found, starting with Ehrlich's Salvarsan against the syphilis agent, which constituted the first efficient and specific therapy for an infectious disease. A quarter of a century later, the sulfa drugs were introduced against many pathogenic bacteria and, in the nineteen forties, the use of penicillin and streptomycin started the exceptionally spectacular and successful new way of treating bacterial diseases; the era of antibiotics had begun.

It was only natural that up to the first half of the present century most bacteriologists be interested first and foremost in the battle against infectious agents and chose the field of medical microbiology. Before Pasteur, infectious diseases were by far the most important cause of death, even in advanced countries. Medical bacteriology attracted attention and few biologists were interested in bacteria for themselves, their structure and their lifestyle. For nearly a century, bacteriology remained centered around the study of disease-causing germs and the general impression prevailed that bacteria were rather primitive parasites, a biological enemy to be controlled and eliminated. This opinion was so deeply rooted in the minds of biologists and the public, that the significance of the important discoveries of soil bacteriologists at the beginning of the twentieth century was entirely overlooked although they were opening important new biological horizons.

2. Progressive discovery of generalized solidarity among prokaryotes.

a) Mixed communities with division of labor

In soil, bacteria proved to be remarkably organized in local communities of mixed types of specialized strains, practicing among themselves and with other microorganisms (e.g. fungi) an efficient division of labor. Soil bacteria were also shown to preserve or improve the fertility of the soil, which caused microbiology to become a subject in the agronomy curriculum. The different types of bacteria were so interdependent that many of them could not be isolated in the laboratory and cultured on artificial media as was successfully done with most pathogenic bacteria. Years later, some soil bacteriologists began to view the close associations and reciprocal support among the bacteria they were studying as comparable to those existing in a multicellular organism (Goret and Joubert, 1949). The contrasting perception that the early twentieth century biologists had about different infectious bacterial species (of medical importance) studied as laboratory entities evidently did not fit well with soil bacteriology. The latter dealt with an assemblage of cooperating, interdependent types like those that exist in many habitats: the soil of prairies, of forests, in lake mud, on the ocean floor, etc. This was a completely novel idea which many bacteriologists chose to ignore and some even to oppose. But soon it was discovered that the digestive tract of all animals carry millions and sometimes billions of bacteria per gram of contents, there again practicing division of labor and often living in a mutualistic or symbiotic relationship with the type of animal that carries them (Drasar and Barrow, 1983; Raibaud and Ducluzeau, 1984). One such complex, very important and well studied association is the one known to exist in ruminants.

In these animals an extremely efficient bacterial mixture of different and typical strains collaborates with unicellular euka-

ryotes (protozoa) to digest cellulose and produce assimilable sugars, short-chain fatty acids and proteins for the plant-eating animals. This microbial association is so well adapted to life in solidarity that no adequate experimental imitation system of the rumen has yet been devised and proven entirely satisfactory. Cattle, camels, sheep, goats, yaks and domestic buffaloes, as well as dozens of wild species are ruminants. Their dependence on stable and complex bacterial associates provides additional proof of the social nature and role of many members of the bacterial population and the complexity and specificity reached by bacterial communities of the digestive tract of animals. Nonetheless, and partly due to the narrow focus of most bacteriological studies, several books continue to present bacteria as individualistic species. But, the situation is evidently more complex in nature and, recently, marine biologists have discovered similar large associations of different bacterial types on the floors of the oceans, at the surface of lagoons, in calm ocean indentations, etc. The development of powerful tools and techniques (gene probing, sequencing, hybridization, fingerprinting) has recently added the enormous number of bacteria living in aquatic habitats to those of the soil and the digestive tract, as specialized members of efficient local associations.They are diverse but are, nonetheless, social strains, working together as temporary and adaptable communities to whose development and survival a successful division of labor is fundamental. It is remarkable how such complex teams can develop wherever a favorable niche appears (Bull and Slater, 1982) and how they can reorganize opportunistically, changing the type of association when needed. Microbial ecosystems are known to be present even in the Earth's subsurface (Krumholz, 1998). Today we know that the pathogenic, infectious bacteria constitute just a very small minority of the bacterial world, and that their parasitic and self-centered habits make them atypical compared with non-pathogenic, community-based strains. However, even with pathogenic bacteria interesting col-

laborations can be observed in mixed cultures *in vitro* and *in vivo* (de Repentigny and Mathieu, 1974, 1976; Lebrun *et al.*, 1978; Mathieu *et al.*, 1976).

b) Horizontal gene exchanges

Solidarity is also manifested in prokaryotes by horizontal gene exchanges that were first seen as exceptional events but which are now known to be generalized and frequently used mechanisms.

In the early forties, studies with the electron microscope had proved that prokaryotes have no nuclei, thus differing markedly from the eukaryotes and confirming Chatton's (1937) concept of the prokaryotes-eukaryotes division of the living world. However, biologists dit not generally consider bacteria as different from other cells. Gradually, new observations showed a clear-cut originality in the structural organization and even the functioning of the genes in prokaryotes.The realization of an authentic, profound difference between the genetics of bacteria and that of eukaryotes progressively set in and gave rise many years later to a new, more realistic concept of the nature of bacteria. It had started in 1928 with Griffith's discovery of transformation (Griffith, 1928). His observations had showed that even dead bacteria could send genes which transformed the hereditary properties of different receptive strains. The change was permanent and inherited but one would have to wait until 1944 to know the nature of the substance (DNA) responsible for the transformation (Avery *et al.*, 1944). Griffith's important discovery was possible because some pneumococci have strains which can kill mice even when fewer than ten living cells are injected intraperitoneally. Since transformation modifies only about one or two bacteria in a million in the conditions of such an experiment, most of the other types of bacteria unable to multiply in the intraperitoneal cavity of mice would have been unsuitable for this kind of finding. They would have been rapidly eliminated by the natural defense systems of mice. But, with the Pneumococ-

cus, if transformation occurred at all, the transformed *virulent* pneumococcal cells were in fact selected by the mice's incapacity to control their replication and deadly invasion. The rapid death of the infected mice became a sure sign that transformation had occurred. Failure to reproduce Griffith's results with other bacterial systems in the next few years led to the erroneous conclusion that transformation was possible only with the Pneumococcus. The phenomenon was considered an oddity and its importance was neglected.

Fifteen years later, penicillin and streptomycin had been discovered and were used as extremely efficient drugs against many bacterial infectious diseases. Shortly afterwards, other antibiotics were found and helped save numerous humans and animals from infections. But, as the use of these wonder drugs was expanding quantitatively and geographically, the number of pathogenic bacteria showing resistance to them began to increase. This created an unexpected and serious public health problem but at the same time offered geneticists the possibility to use bacterial drug resistance as an experimental tool. In laboratory experiments, it afforded a powerful selection agent for bacteria that had received the resistance gene(s): they could simply be grown on a culture medium containing the respective antibiotic(s). Only the transformed cells carrying the transferred resistance gene(s) could grow under such conditions. In this way more types of bacteria (considered then different species) were shown to be able to become competent and perform transformations. Each time a new type was successfully transformed in the laboratory, the list of transformable bacteria grew accordingly but this did not lead to rapid general acceptance of transformation as an important genetic mechanism for the horizontal transfer of genes among bacteria in nature. The old prejudices were not dying easily. Nowadays indications are that transformation can occur in about half of the studied bacterial strains but its real importance remains underestimated.

Following the discovery of transformation, intriguing reports on the (then mysterious) frequent mechanisms for horizontal gene transfer in prokaryotes began to appear, in the late nineteen forties and extending over into the fifties. These gene transfer processes involve the exchange of small replicons, i.e. self-replicating DNA molecules (other than the much larger, stable replicon carrying the essential genes and also called nucleoid or chromosome). Small replicons are present on a temporary basis in any known prokaryotic cell. Their copies are easily exchanged with and accepted by other strains. The small replicons may also carry a few stable genes from the large replicon of the donor cell or other genes received previously from other donors. These are *converting*, occasionally useful, accessory genes. Prophages and self-transmissible (ST) plasmids (also called conjugative plasmids) are the most advanced forms of small replicons. Other genes from the donor cell can be carried along and transferred into the receptor cell by these two types of ST small replicons. Most of these genes originate from the large replicon of the donor but some may come from its small replicons. The theoretical and practical importances of these surprising discoveries on basic bacterial genetics have been acknowledged and some of their authors rewarded by the Nobel prize: for example, Joshua Lederberg and his group, André Lwoff and François Jacob. However, these discoveries seemed then to be of rather limited importance in nature. They were considered rare occurrences without real significance for bacterial life in general.

The first indication that genes transferred horizontally by small replicons from one bacterial strain to another could play significant roles in nature was Freeman's discovery, in 1951, that a visiting prophage was necessary for the diphtheria bacillus to produce, experimentally or spontaneously, the diphtheria toxin, a strong poison responsible for a serious, sometimes deadly illness. The toxin is encoded by a virulence gene carried by the prophage. Within a short period of time, similar virulence con-

versions were proven for different pathogenic bacteria and their prophages (Ackermann and DuBow, 1987). The subject soon attracted many able investigators and before a decade several ST plasmids had also been shown to harbor virulence genes responsible for illnesses caused by other pathogenic bacteria (Broda, 1979; Falkow, 1997). This series of results proved for the first time that small replicons are very important even in the pathogenic bacteria's way of life which, as parasites of eukaryotes, do not participate in bacterial teams. Metabolic conversions (Gunsalus et al., 1975) also probably exist on a large scale but are hard to discover because of the lack of an easy laboratory procedure to select for the converted cells; this latter type of gene exchange therefore has not yet been readily accepted as an important mechanism in bacterial life.

By the early 1950's, resistance of pathogenic bacteria to antibiotics was widening in scope and increasing in frequency. For nearly twenty years it was supposed that this alarming, unforeseen development was due to mutations, but the results of numerous studies undertaken to test this hypothesis always failed to support it. Japanese microbiologists (Watanabe, 1963) presented the first evidence that ST plasmids played a crucial role in the dissemination of antibiotic resistance among pathogenic bacteria. These plasmids were shown to be able to transfer drug resistance genes, the latter probably originating from the soil bacteria. The Japanese scientists' results were confirmed in numerous experiments performed in different countries. Soon it was found that prophages too could play a similar role in the dissemination of resistance (Richmond and John, 1964). As a consequence, the ability of ST plasmids and of prophages to transfer resistance to one or several antibiotics, a frequent and important phenomenon, has been accepted by all biologists. The fact that the genes responsible for resistance to toxic substances have been, very probably, obtained from soil bacteria lends further support to our conviction that even pathogenic bacteria do not operate in a

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