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Invitational ONR Lecture

Bacterial Structure in Today's Research

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The microscope and all levels of resolution are fundamental to the study of microbes; it is true that beer was brewed and cheese ripened long before the advent of the comforting benefit of microscopic control, as has been the case of many a sophisticated fermentation. But visual recognition of the varieties of microbes in the first half of the 19th century made for a remarkable and quantum change in attitude and control; no less extraordinary developments followed the evolution of biochemistry and the increased resolution of electrons over photons attained in the first half of the 20th century. What has been added to this amplification of the senses is a remarkable elaboration of human perception: the correlation of structural, biochemical, and functional information as a natural part of looking at all kinds of cells including microbes so that microscopy is once again at the frontiers of biological research. Now, one can hardly look at a micrograph of any type of cell without an automatic interpretation of structure in biomolecular terms. Despite the crudity and limitations of cytochemical techniques applicable to microscopy, the strength of microscopy is in the evocative nature of visual images and the complexity of molecular and physiological organization that is tied up in structure, even the most delicate.

Some of us still study cell-structure for its own sake but, much of the time, the practical realities of attaining research support or maintaining credibility in our varied human endeavors enforces an interface with practical-minded colleagues and their problems. These associations must have been productive and of some satisfaction over the years to all concerned. Imitation is the sincerest form of flattery and it is now clear from a not too casual inspection of microbiological journals that a high proportion of papers display or refer to definitive cytological information and involve sophisticated microscopy. The involvement does not concern only descriptive and determinative functions, but includes principles for the assembly and association of macromolecules to make structures, the correlation of structure and function, the effects of drugs and viruses on the metabolic organelles, growth and division of bacteria, the details of differentiation and life cycles, and cell-cell interactions. In many cases the judicious use of microscopy draws attention to the nature of a problem and becomes an essential feature of analysis and control, whether we are talking of a microbiological process or a research problem.

Everyone develops a set of prejudices and a *modus vivendi* suited to his talents and his "solvable" problems; microscopists are not immune to this narrowing of focus. My

own interests have revolved around the structure of the bacterial cell envelope and, particularly, the cell wall. This was both fortuitous and fortunate: the cell wall is accessible to study with a wide range of techniques and, in justification, is involved in dynamic and essential cellular processes so that the interests and prejudices of a microscopist are parallelled by those of the practitioners of many of the other "black arts" of modern biology. It is now recognized by those manipulating and studying ribosomes, nucleoplasms and plasmids, membranes, or the whole cell in growth and division that a variety of techniques and the highest standards of microscopy are a necessity and not a luxury.

The description of a bacterium for purposes of formal classification must include structure at sufficient resolution to confirm that it indeed has the characteristics of a procaryote as well as any special features that facilitate its placement within the hierarchy. This draws attention to the remarkable potency of structural characters which are critical in assessment at both the level of kingdom and the level of genus and sometimes species. The mutants that are generated so easily today may involve complex temperature-sensitive aberrations of systems and not infrequently these have morphogenetic expression. They provide varied opportunities to illuminate and to test our structural models. Formal speciation almost requires that the organism be cultivable, but there are a remarkable number of endosymbiotic procaryotes in a wide range of cells of protists, plants, and animals that can be recognized only for what they are by the sophisticated techniques of microscopy and molecular biology since they are not yet cultivable. One of the remaining frontiers of microbiology lies in developing the capability to study these associations in a more than superficial manner.

Good descriptions and well-thought-out assessments of characters for determinative purposes are essential to most if not all bacteriologists. One must be able to recognize "old friends," to categorize or classify the new, and to assess relatedness. It is somewhat humbling to remember that the most common determinative errors occur at the very beginning of the process: shape, color (i.e., Gram reaction), and motility. The fancy techniques of microscopy come later (or never) and can play a modifying role by assessing the form of the wall profile as a control to the Gram reaction, and the presence or absence of flagella or certain types of pili in respect of motility. Planes of division and some aspects of shape and form may well be illuminated by both scanning and transmission electron microscopy. But it is still true that effective use of the light microscope is the critical factor in good determination.

Structural principles have proved hard to gain and slow to integrate into our overall understanding. At one point it was enough to know what sorts of macromolecules were included in a structure, then it became necessary to know where they were (and their associations) in the structure, and how the components were made and, in some cases, exported. Now we ask questions about assembly and the conditions required for this and for any differentiation to take place. It is becoming clear to us that the assembly of a structural feature of the cell is, in essence, an exercise in physical chemistry although subsequent maturation, modification, and differentiation may be mediated by enzymes. The predominately anionic macromolecules require a suitable environment to modify charge-density and, perhaps, shape so that they can become closely associated for self-assembly (or entropy directed assembly). This latter may be mediated by small, usually divalent cations as well as the steric arrangement of reactive groups. It is then

possible, as is often true but not inevitable, that covalent bonding is established with the assistance of appropriate enzymes.

The classical models for self-assembly structures have been provided by the assembly of viruses within cells and, given the essential materials, in the test tube. It is now apparent that we have an easily accessible set of models provided by bacterial cell walls and increasing understanding of assembly and functional association of macromolecules in even more complex structures such as the plasma membrane.

One puzzling feature of bacterial cell walls has been the complexity of the structural profile with the obvious and regular layering of components, so clearly demonstrable in gram-negative bacteria. Not only is there sorting of sets of macromolecules but the order is definite and immutable. They are assembled outside of the plasma membrane and the polymeric components have to be exported by the cell in fairly complex form. Conventionally, the innermost layer is the peptidoglycan or murein network. This may be interlocked with teichoic or teichuronic acid polymers in gram-positive bacteria, or, in some gram-negative bacteria, may be covalently linked with a lipoprotein which is neatly enmeshed in the complex protein-lipid-lipopolysaccharide-protein complex forming the outer membrane. Many bacteria, both gram-negative and gram-positive, have regular layers outside of these basic elements of the cell wall and in many cases these include one or more layers of macromolecules (mostly protein in nature) in paracrystalline arrays. It is these outermost components that are the most amenable to study as self-assembly systems. Of course, we would like and need to know all we can about the structure and assembly of the peptidoglycan layers for practical purposes but microscopy does not yet provide useful input into models of structure and arrangement.

The important things that we have learned from these regularly structured wall layers are: (1) they may consist of a single polymeric species; (2) they can be assembled onto "naked" cells or in vitro to give the precise pattern of the original material; (3) assembly requires a suitable cationic environment and often has an absolute requirement for specific divalent cations (usually Ca^{2+}); and (4) some assemblies require a suitable nucleation surface provided by the layer beneath in the wall profile. If this can be carried further in both principle and in actual models, it should not be surprising that walls and other structures have definite orders and arrangements; they are dictated by the requirements for assembly and the physico-chemical behavior of the component polymers.

It is now apparent that the arrangement of the components of both the outer membrane as well as the physiologically critical plasma membrane is not symmetrical and, also, that there must be area differentiation for these structures to function. Cleavage of frozen membranes and replication of the exposed surfaces does tell something about the distribution and behavior of the protein macromolecules in this protein-lipid-protein environment. Equally, studies of induced synthesis of new products (such as a lipopolysaccharide) gives a feeling for area specialization and coordination (e.g., in "adhesion zones"). One cannot doubt that assembly plays a role in the constitutive and adaptive functions of such structures.

The functional aspects of structure are, then, important in the integration of modern microscopy with the many facets of bacterial biochemistry. An area of major interest, among many, concerns cell wall behavior during growth and division because a rather rigid enclosure has to be expanded in such a way as to maintain or perpetuate cell

shape without loss of integrity. The products of division, the sister cells, must be separated by a cell wall septum, which is built or differentiated in such a way that separation can occur. Cell growth is attained not by general intercalation but by closely regulated zones of synthesis initiated by signals during replication of the chromosome. In some cases, especially cocci, the synthesis and septum formation are essentially functions of the same system involving septal synthesis and outflow from that site. Others, particularly the rod-shaped bacteria, must have separable elongation and septation functions in order to maintain shape. So mutations occur that are deficient or altered in septation without affecting elongation. Likewise, some chemicals and antibiotics discriminate between these functions. Many but not all the chemical and physical treatments leading to aseptate filaments do so by interference with DNA replication. However, other forms of interference are possible such as with critical enzymes (especially the carboxypeptidase-transpeptidases) involved in cross-linking peptidoglycan and which bind the β-lactam antibiotics. Microscopy assists in these complex studies by supplying structural markers and description of the order of events in the process of cell division. Unfortunately, it is apparent that individual layers of cell wall follow different rules of procedure, e.g., it is commonly observed that cell septation involves the peptidoglycan-rich layer and obvious synthesis and participation of external layers (such as outer membrane of gram-negatives) is a late event occurring when separation is in progress. Structural markers, specific bacteriophages, fluorescent antibody, and pulse-labeled autoradiographs assist the microscopic determination of the order and topology of events.

The intricacies of swimming motility have enjoyed extraordinary attention because microscopy has revealed the remarkable infrastructure of the basal complex that integrates flagella into wall and plasma membrane. Direct observation has provided the stimulus for concepts of a mechanism involving a proton-motor-driving rotation of the semirigid helixes that are the flagella. Although many details are still unresolved, this is a fascinating area for the study of structure and function at the highest levels of resolution.

An unexpected outcome of electron microscopy of the arrangement and assembly of wall polymers concerns the distribution and behavior of metal cations in biological polymers. Electron microscopy depends on scattering of electrons by the heavier atoms in the structure being examined. In the sections that give information about layering or differences in regions of a structure, this is due to the differential incorporation of metals included in fixation or postfixation treatments, including "staining" of the sections after cutting, using solutions containing heavy metals. It is obvious that structures trap a variety of metals. Assembly, as pointed out, usually requires divalent cations and often exhibits extreme specificity in this requirement. Many organisms (plants and animals as well as procaryotes) accumulate trace or rare elements from their environments. Modest experiments show that durable biological polymers such as those represented in bacterial cell walls have varied interactions with a long list of metal salts. Some are taken up avidly, others not at all; some are strongly bound, others are easily displaced; some are very efficient at displacement and others are not. Given access to a broad mixture of metal salts, a great diversity of metals is trapped. These phenomena may explain how bacteria (and, indeed, the wide spectrum of life) can accumulate and hold onto the trace elements needed for specific enzymic processes and are protected to a degree from lower concn of those that are toxic. This is a more general situation

than the enterochelins that allow some bacteria to compete with their hosts for the iron that they need for metabolic health. There is then an extension of the functional capacities of the polymers that envelop cells of various kinds or envelop complex organisms including plants and animals, and which may play important roles in nature in the transport of metals. Some of the ideas are old, such as "contact ion exchange" for plant roots, but the overall view is only now being established.

Differentiation is a critical characteristic of life and even of procaryotic cells, which may seem to have limitations of scope as well as complexity. This is illusory and even the "simplest" systems pose formidable questions. In most situations (ranging from unequal cell division and budding to extreme dimorphism, sporulation, and fruiting-body formation), the microscopic parameters are critical to monitoring the biochemical-molecular intricacies, to classifying mutants, and to recognizing where the problems lie.

Among the differentiations that have significant input for research today are those that are of concern to a sessile life and interactions with surfaces or other cells. This may involve the specialized areas of surfaces forming holdfasts, the more general attributes of piliation that mediates cell-cell attachment or the capsular polymers that entrap cells and are part of cell-substrate attachment. The mechanisms are important because the resulting behavior is ecologically significant, ranging from dental plaque and associations critical to colonization and disease to the precarious life on solid interfaces in natural waters. We need the stimulus of thinking about such phenomena, which put the trivial irritations of wall growth and aggregation in chemostat fermenters into perspective!

The massive clones of procaryotic life have been challenging nature with a myriad of small experiments for over 3 billion years. No doubt some of their innovations so derived, and those of their unknown ancestors, have been successful enough to survive and some are so universally a part of life because of spectacularly effective qualities. We still only guess at the actual dynamic and protean working structure of the plasma membrane, which is *the* organelle of the procaryotic cell, and admire its suitability for a multitude of simultaneous tasks. An industrial microbiologist might well hope to emulate as well as take advantage of procaryotic systems.

"The path of science is not such that only
one man can tread it at a time."
Francis Bacon

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