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CONTENTS/SUMMARIES

- There Must Be a Prokaryote Somewhere: Microbiology's Search for Itself.** Carl R. Woese 1-9

Summary: While early microbiologists showed considerable interest in the problem of the natural (evolutionary) relationships among prokaryotes, by the middle of this century that problem had largely been discarded as being unsolvable. In other words, the science of microbiology developed without an evolutionary framework, the lack of which kept it a weak discipline, defined largely by external forces. Modern technology has allowed microbiology finally to develop the needed evolutionary framework, and with this comes a sense of coherence, a sense of identity. Not only is this development radically changing microbiology itself, but also it will change microbiology's relationship to the other biological disciplines. Microbiology of the future will become the primary biological science, the base upon which our future understanding of the living world rests, and the font from which new understanding of it flows.

- Lessons from an Evolving rRNA: 16S and 23S rRNA Structures from a Comparative Perspective.** Robin R. Gutell, Niels Larsen, and Carl R. Woese. 10-26

Summary: Underlying the functional complexity of large macromolecules, such as the rRNAs, is their structure. Underpinning the complex structure of the rRNAs is a fundamental set of RNA structure principles that transforms a sequence of nucleotides into an intriguing and puzzling assemblage of RNA structure. Currently, our understanding of these principles of RNA structure is rudimentary. Utilizing those known to transform a primary structure into its higher-order structure, while improving, remains far from an exact science. The 16S and 23S rRNAs, the focus of these studies, have, like other biological entities, evolved to their present state. The process of mutation and selection has molded these higher-order structures into their present forms. Although we do not necessarily understand the pathways or the selection pressures of this evolutionary process, analysis of a large collection of structurally homologous 16S (and 16S-like) and 23S (and 23S-like) rRNAs is quite revealing. The secondary and other higher-order structure for these large RNA molecules, over 1,500 and 2,900 nucleotides in length, respectively, are presented herein, along with a few emerging principles of (r)RNA structure.

- Anaerobic Bacteria from Hypersaline Environments.** Bernard Ollivier, Pierre Caumette, Jean-Louis Garcia, and Robert A. Mah 27-38

Summary: Strictly anaerobic halophiles, namely fermentative, sulfate-reducing, homoacetogenic, phototrophic, and methanogenic bacteria are involved in the oxidation of organic carbon

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in hypersaline environments. To date, six anaerobic fermentative genera, containing nine species, have been described. Two of them are homoacetogens. Six species belong to the family Haloanaerobiaceae, as indicated by their unique 16S rRNA oligonucleotide sequences. *Desulfohalobium retbaense* and *Desulfovibrio halophilus* represent the only two moderately halophilic sulfate reducers so far reported. Among anoxygenic phototrophic anaerobes, a few purple bacteria with optimal growth at salinities between 6 and 11% NaCl have been isolated from hypersaline habitats. They belong to the genera *Rhodospirillum*, *Chromatium*, *Thiocapsa*, and *Ectothiorhodospira*. The commonest organisms isolated so far are *Chromatium salexigens*, *Thiocapsa halophila*, and *Rhodospirillum salinarum*. Extremely halophilic purple bacteria have most commonly been isolated from alkaline brines and require about 20 to 25% NaCl for optimal growth. They belong to the family *Ectothiorodospiraceae*. Their osmoregulation involves synthesis or uptake of compatible solutes such as glycine-betaine that accumulate in their cytoplasm. The existence of methanogens in hypersaline environments is related to the presence of noncompetitive substrates such as methylamines, which originate mainly from the breakdown of osmoregulatory amines. Methanogenesis probably does not contribute to the mineralization of carbohydrates at NaCl concentrations higher than 15%. Above this concentration, sulfate reduction is probably the main way to oxidize H₂ (although at rates too low to use up all the H₂ formed) and occupies a terminal function in the degradation of carbohydrates. Three genera and five species of halophilic methylotrophic methanogens have been reported. A bloom of phototrophic bacteria in the marine salterns of Salins-de-Giraud, located on the Mediterranean French coast in the Rhone Delta, is also described.

Molecular Genetics of *Thiobacillus ferrooxidans*. Douglas E. Rawlings and Tomonobu Kusano..... 39–55

Summary: *Thiobacillus ferrooxidans* is a gram-negative, highly acidophilic (pH 1.5 to 2.0), autotrophic bacterium that obtains its energy through the oxidation of ferrous iron or reduced inorganic sulfur compounds. It is usually dominant in the mixed bacterial populations that are used industrially for the extraction of metals such as copper and uranium from their ores. More recently, these bacterial consortia have been used for the biooxidation of refractory gold-bearing arsenopyrite ores prior to the recovery of gold by cyanidation. The commercial use of *T. ferrooxidans* has led to an increasing interest in the genetics and molecular biology of the bacterium. Initial investigations were aimed at determining whether the unique physiology and specialized habitat of *T. ferrooxidans* had been accompanied by a high degree of genetic drift from other gram-negative bacteria. Early genetic studies were comparative in nature and concerned the isolation of genes such as *nifHDK*, *glnA*, and *recA*, which are widespread among bacteria. From a molecular biology viewpoint, *T. ferrooxidans* appears to be a typical member of the proteobacteria. In most instances, cloned gene promoters and protein products have been functional in *Escherichia coli*. Although *T. ferrooxidans* has proved difficult to transform with DNA, research on indigenous plasmids and the isolation of the *T. ferrooxidans merA* gene have resulted in the development of a low-efficiency electroporation system for one strain of *T. ferrooxidans*. The most recent studies have focused on the molecular genetics of the pathways associated with nitrogen metabolism, carbon dioxide fixation, and components of the energy-producing mechanisms.

Control of Meiotic Gene Expression in *Saccharomyces cerevisiae*. Aaron P. Mitchell..... 56–70

Summary: Sporulation of the yeast *Saccharomyces cerevisiae* is restricted to one type of cell, the a/α cell, and is initiated after starvation for nitrogen in the absence of a fermentable carbon source. More than 25 characterized genes are expressed only during sporulation and are referred to as meiotic genes or sporulation-specific genes. These genes are in the early, middle, and late expression classes. Most early genes have a 5' regulatory site, URS1, and one of two additional sequences, UAS_H or a T₄C site. URS1 is required both to repress meiotic genes during vegetative growth and to activate these genes during meiosis. UAS_H and the T₄C site also contribute to meiotic expression. A different type of site, the NRE, is found in at least two late genes. The NRE behaves as a repression site in vegetative cells and is neutral in meiotic cells. Many regulatory genes that either repress or activate meiotic genes have been identified. One group of regulators affects the expression of *IME1*, which specifies a positive regulator of meiotic genes and is expressed at the highest levels in meiotic cells. A second group of regulators acts in

parallel with or downstream of *IME1* to influence meiotic gene expression. This group includes *UME6*, which is required both for repression through the *URS1* site in vegetative cells and for *IME1*-dependent activation of an upstream region containing *URS1* and *T₄C* sites. *IME1* may activate meiotic genes by modifying a *UME6*-dependent repression complex at a *URS1* site. Several additional mechanisms restrict functional expression of some genes to meiotic cells. Translation of *IME1* has been proposed to occur only in meiotic cells; several meiotic transcripts are more stable in acetate medium than in glucose medium; and splicing of *MER2* RNA depends on a meiosis-specific gene, *MER1*.

Computer-Aided Analyses of Transport Protein Sequences: Gleaning Evidence concerning Function, Structure, Biogenesis, and Evolution. Milton H. Saier, Jr.

71-93

Summary: Three-dimensional structures have been elucidated for very few integral membrane proteins. Computer methods can be used as guides for estimation of solute transport protein structure, function, biogenesis, and evolution. In this paper the application of currently available computer programs to over a dozen distinct families of transport proteins is reviewed. The reliability of sequence-based topological and localization analyses and the importance of sequence and residue conservation to structure and function are evaluated. Evidence concerning the nature and frequency of occurrence of domain shuffling, splicing, fusion, deletion, and duplication during evolution of specific transport protein families is also evaluated. Channel proteins are proposed to be functionally related to carriers. It is argued that energy coupling to transport was a late occurrence, superimposed on preexisting mechanisms of solute facilitation. It is shown that several transport protein families have evolved independently of each other, employing different routes, at different times in evolutionary history, to give topologically similar transmembrane protein complexes. The possible significance of this apparent topological convergence is discussed.

Gas Vesicles. Anthony E. Walsby.

94-144

*Summary: The gas vesicle is a hollow structure made of protein. It usually has the form of a cylindrical tube closed by conical end caps. Gas vesicles occur in five phyla of the Bacteria and two groups of the Archaea, but they are mostly restricted to planktonic microorganisms, in which they provide buoyancy. By regulating their relative gas vesicle content aquatic microbes are able to perform vertical migrations. In slowly growing organisms such movements are made more efficiently than by swimming with flagella. The gas vesicle is impermeable to liquid water, but it is highly permeable to gases and is normally filled with air. It is a rigid structure of low compressibility, but it collapses flat under a certain critical pressure and buoyancy is then lost. Gas vesicles in different organisms vary in width, from 45 to >200 nm; in accordance with engineering principles the narrower ones are stronger (have higher critical pressures) than wide ones, but they contain less gas space per wall volume and are therefore less efficient at providing buoyancy. A survey of gas-vacuolate cyanobacteria reveals that there has been natural selection for gas vesicles of the maximum width permitted by the pressure encountered in the natural environment, which is mainly determined by cell turgor pressure and water depth. Gas vesicle width is genetically determined, perhaps through the amino acid sequence of one of the constituent proteins. Up to 14 genes have been implicated in gas vesicle production, but so far the products of only two have been shown to be present in the gas vesicle: *GvpA* makes the ribs that form the structure, and *GvpC* binds to the outside of the ribs and stiffens the structure against collapse. The evolution of the gas vesicle is discussed in relation to the homologies of these proteins.*