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MOLECULAR BIOLOGY OF MEMBRANE-BOUND COMPLEXES IN PHOTOTROPHIC BACTERIA

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PREFACE

Cells of phototrophic bacteria are fitted out with a characteristic and sometimes species-specific membrane-system: the continuous but differently composed cytoplasmic-intracytoplasmic membrane systems in purple bacteria, the cytoplasmic membrane with the attached light-harvesting chlorosomes in green bacteria and the intracytoplasmic thylakoids with the attached light-harvesting phycobilisomes in cyanobacteria.

During the long-lasting evolutionary process phototrophic bacteria have been adapted to numerous ecological niches and on this way they have developed various types of light-harvesting antenna systems and pigments. The evolutionary pressure on the development of efficient energy-transducing systems resulted, on the other hand, in homologous structures with a high similarity of primary amino-acid sequences of membrane-bound pigment-binding polypeptides and very similar principles of organization, realized, for example, in the photochemical reaction center and the ubiquinone-cytochrome b/c_1 oxidoreductase of many evolutionary remote organisms.

The bacterial photosynthetic and respiratory apparatuses are much simpler in composition and organization than the corresponding structures of higher organisms. They are, therefore, excellent model systems to study correlations between structure and function and assembly of these highly organized membrane particles. Biophysicists, biochemists and molecular biologists have in close cooperation, but using different methodical approaches, reached a clear progress in this field.

From the 150 contributions to the Symposium on molecular biology of membrane-bound complexes in phototrophic bacteria (Freiburg, August 2-5, 1989) 56 representative papers have been selected and combined in this volume.

The first group of articles is dedicated to structure and regulation of genes coding for pigment-binding membrane proteins and enzymes for bacteriochlorophyll, carotenoid and cytochrome synthesis under the control of oxygen and light gradients.

Articles of the second chapter deal with composition, structure, organization and function of membrane-bound protein complexes. The localization, orientation and binding of pigments to proteins, the structure of polypeptides in the membrane, the process of excitation energy transfer in antenna complexes, the charge separation and quinone reduction in reaction centers and the reconstitution of the complexes from their subunits are some of the major features.

The third chapter summarizes progress in the analysis of different electron transport systems, the formation of gradients of charges and ions across membranes, specific transport systems, chemotactic sensory transduction and excitation of the flagellar motor.

All those who are interested in structure, function and formation of membrane-bound complexes will find facts and stimulating hypotheses in this field.

I wish to thank the Deutsche Forschungsgemeinschaft and Federation of European Microbiological Societies for financial support and all friends and colleagues who contributed to the success of the meeting.

Gerhart Drews

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This introductory chapter provides an overview of the topics currently studied in photosynthetic bacteria with the tools of molecular genetics, and comments on the appropriate matching of tools and tasks.

Photosynthetic bacteria are a diverse assemblage of microorganisms, distributed over a variety of different habitats and possessing a correspondingly wide range of metabolic activities. Historically, the primary motivation for the study of these bacteria has been to gain a better understanding of photosynthesis itself, but an important secondary theme, which has been explored in many of the photosynthetic bacteria, is the incredible metabolic diversity displayed in the energy metabolism of this group. These dual themes are reflected in the tools of molecular genetics that are being applied to photosynthetic bacteria today. On the one hand, site-directed mutagenesis is being used extensively to probe the structure/function relationships of photosynthetic reaction centers and other energy transduction systems, while studies of the regulation of gene expression, employing the full range of molecular genetic techniques, seek to understand how organisms adapt to changing environmental conditions.

MOLECULAR GENETICS IN STRUCTURE/FUNCTION STUDIES

The basic paradigm for structure/function analysis of reaction centers or antennae by site-directed mutagenesis was published by Youvan et al. in 1985. These authors deleted the chromosomal copies of the reaction center genes, and then supplied engineered copies of those genes on plasmids. In this way they could relatively quickly generate sets of amino acid substitution mutations at points of interest in the reaction center or antenna proteins, allow the altered proteins to be synthesized, and analyze the phenotype of each. They adopted a powerful way of displaying the data generated from these experiments (Bylina and Youvan, 1987). The standard set of twenty amino acids are graphed in two dimensions by plotting molar volume vs hydrophobicity for each, and then contours are drawn around groups of amino acids that may be substituted at a given position to give a particular phenotype (herbicide resistance, photosynthetic competence, etc.). These plots typically give simple contours, suggesting that in many cases these two parameters are sufficient to describe the important features of a residue that determine its function.

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It will be recognized from the above example that three genetic capabilities are essential for experimentation using site-directed mutagenesis. First, the relevant genes must be cloned. This capability is generally not a barrier to work with any species, but it is easier to work with organisms for which gene banks are readily available. DNA is usually cloned into *Escherichia coli,* and site directed mutagenesis conducted there by any of a variety of available techniques. Second, it is usually necessary to return engineered DNA to the species of origin. For the deletion/complementation paradigm described above, this requires replicons that are more or less stably maintained in the species of origin as well as in *Escherichia coli,* and carry selectable genetic markers, usually antibiotic resistance-conferring genes. Furthermore, a mechanism must exist for depositing DNA in the cytoplasm of the original species. Here the biology of the individual species varies widely, and some organisms are much better genetic hosts than others. For example *Rhodopseudomonas viridis* has proven most intractable as a genetic research object. Another factor that distinguishes the suitability of an organism for genetic studies is not a biological distinction but an historical truism. Organisms that have been previously used for genetic studies are better objects for additional genetic studies than virgin organisms, largely because of the size of the genetic tool kit available for each species. Third, there must be a convenient means for precise manipulations of regions on the chromosome of the species of origin. For the deletion/complementation paradigm, this can be accomplished by returning to the species of origin an engineered DNA segment in which a region of DNA has been replaced by a selectable marker, and then identifying clones in which homologous recombination has lead to a replacement of the chromosomal region in question by the engineered DNA. This is most easily accomplished in *R. capsulatus,* using the gene transfer agent-mediated technique developed by Scolnik and Haselkorn (1984). A different strategy for chromosomal engineering is required for other species, which do not have a system for delivering linear DNA fragments like those carried by the gene transfer agent of *R. capsulatus.* It is possible to introduce chromosomal deletions plasmids that cannot replicate in the target organism, but the resulting clones must be carefully screened to determine their true genotype, since a variety of genetic events other than homologous replacement can occur. It is conceivable that site-directed mutations could be directly introduced onto the chromosome, avoiding the deletion/complementation paradigm, but tools to accomplish this easily and reproducibly do not currently exist. Ease and reproducibility are important requirements for such tools, because thorough site-directed studies require the construction of not just one new mutation, but many changes at each site of interest. Tools for this purpose are under development in *R. capsulatus.*

"O, the June Bug she has golden wings, the Lightning Bug has fame; the Weevil has no wings at all, but he gets there just the same" (from an American folk song). The superior genetic systems of *Rhodobacter capsulatus* allowed the first cloning, complete sequencing and site-directed changes of photosynthetic reaction center genes, but reaction center crystals were first made, and their structures solved, with material isolated from *Rhodopseudomonas viridis. Rhodobacter sphaeroides* placed second in both of those races, but it is currently the only organism for which we have both crystal structure data and genetic systems capable of site-directed mutagenesis, so an increasing amount of structure/function work is being done in that system. Although crystal structures for the reaction centers of *R. capsulatus* are not available, it has been possible to analyze the results of mutational changes in *R. capsulatus* reaction centers by assuming significant structural homology accompanies the obvious amino acid sequence homologies between *Rhodobacter sphaeroides* and *Rhodobacter capsulatus* (78% identity for the L subunits of the *Rhodobacter* species, 77% for their M subunits; Williams et al, 1984). The general validity of this assumption is attested to by the coherence of results from numerous site-

directed changes when interpreted under the assumption of homology. Another way to get around the lack of both good genetics and crystal structure in the same organism would be to move the genes for either *R. sphaeroides* or *Rhodopseudomonas viridis* reaction centers into an *R. capsulatus* deletion strain. There is some evidence that at least the *R. sphaeroides* reaction centers would function properly in the *R. capsulatus* cell milieu (Daldal, personal communication). Less global changes, involving partial swaps of one peptide or peptide segment for another, will test how homologous the proteins from the various species really are, but will probably not simplify the interpretation of functional changes in terms of precise structural data, unless whole new crystal structures are generated.

Cyanobacteria, the blue-green algae with plant-like photosynthetic systems, have more recently gained the minimal set of genetic tools with which site-directed experiments can be conducted. The primary organisms used are *Synechococcus sp.* PCC 7002 and *Synechocystis* 6803. Photosystem II is studied, again making the assumption of homology to crystal structures of the *R. sphaeroides* and *Rhodopseudomonas viridis* reaction centers. The water splitting capabilities of PSII are not found in the simpler purple photosynthetic bacteria, so this is an ideal area of study in which to apply the blue-green systems. Phycobilisomes, the well-characterized antenna system unique to cyanobacteria, are also objects of investigation by site-directed mutagenesis techniques.

MOLECULAR GENETICS IN GENE EXPRESSION STUDIES

There are at least three dimensions that are relevant to a discussion of currents studies of gene expression in photosynthetic bacteria: what physiological systems are under study, in which organisms are they being studied, and at what level of genetic organization are the studies focussed. Nitrogen fixation, pigment biosynthesis, hydrogen metabolism, and electron transport join antennae and reaction center studies as those physiological systems most explored at the level of gene expression in photosynthetic bacteria. In the accompanying papers we find a sampling of a few more genera *(Chloroflexus, Calothrix, Pseudanabaena)* than are used in the structure/function studies discussed above, but the majority of the work is conducted with *Rhodobacter* species or *Synechocystis* or *Synechococcus*. One notes that the purple sulfur bacteria and their remarkable transformations of sulfur compounds are not among the objects of genetics-assisted study. Most unexpected is the range of levels of gene organization over which these questions have been explored, running the gamut from genomic mapping, through superoperons, operons, genes and control regions, right down to individual mutations.

The most widely studied system in the photosynthetic bacteria is the regulation of expression of genes coding for the photosynthetic apparatus itself. The range of regulatory phenomena exhibited in the photosynthetic apparatus of *Rhodobacter* species, for example, includes changes in level of expression in response to oxygen tension, light intensity and carbon source, and changes in the composition of the apparatus in response to these same signals. Throughout these changes, neither free pigments nor free proteins are accumulated to a measurable extent, implying that small molecule and large molecule syntheses are somehow coordinated. Furthermore, assembly and accumulation of one type of antenna complex (light-harvesting II) is normally dependent upon the synthesis of carotenoids, implying a mechanism for coordinating that particular pigment/protein pair.

The availability of genetic tools often determines what studies are carried out in pursuit of understanding, and studies of how regulation of gene

expression occurs fit this pattern. Some tools, such as hybridization-based assays (Southern, northern and dot blotting, S1 mapping etc.) and sequence analysis require only cloning technologies, and thus are applicable to virtually any system. With these techniques an investigator could theoretically measure changes in expression of the level of transcripts from any given gene, and study which genes are co-regulated and co-transcribed with other genes. The difficulty in using this approach alone lies in the variability of the metabolism of mRNA's in bacteria. Some segments of mRNA have short half-lives compared to others, ranging from a matter of seconds to tens of minutes, so the amount of signal measured by hybridization depends on both synthesis and degradation rates. Furthermore, since transcription, translation and degradation are known to proceed simultaneously on the same transcript in bacteria, different genes from the same operon may seldom or never be found on the same molecule of mRNA. Finally, complete knowledge of any hybridization probe is essential. If a restriction fragment used as a probe for a particular gene also carries other genetic regions, misleading data can easily be generated. For these and other reasons, it is a great aid to the interpretation of hybridization data to also have data reflecting gene expression at the translational level in the organism of interest. This may be accomplished by fusion of a reporter enzyme sequence to the genetic region under investigation, a technique that requires methods for introducing engineered DNA into the organism of interest. This is a limitation for many systems, and time consuming for any system, but the data gathered can be critical for understanding.

A description of the *puf* operon provides illustrations of some of these points. This operon includes, in the following order, the *pufQ* gene, required for bacteriochlorophyll synthesis, *pufB* and A, light harvesting I antenna peptides, *pufL* and *M*, reaction center proteins, and *pufX* of unknown function. Early work in *R. capsulatus* (Belasco et al, 1985), based on sequence analyses and S1 nuclease 5' end mapping, suggested that the *puf* operon began just upstream of the *pufB* gene. Functional analysis of this region, using fusions of a reporter enzyme to proteins of the *puf* operon, later showed that the operon actually begins approximately 700 bp upstream from the *pufB* gene, and a new gene, *pufQ* was located in the intervening region (Bauer *et al*, 1988). Similar confusion seems to currently exist in the *R. sphaeroides* system (Kansy and Kaplan, 1989), where workers have attempted to locate the beginning of the *puf* operon by analyzing various types of biochemical data in the absence of appropriate genetic data. They have described a great number of RNA fragments, and they suggest that the *puf* operon begins just upstream of the *pufB* gene. While it remains a logical possibility that *R. capsulatus* and *R. sphaeroides* are fundamentally different in regulation of expression of the *puf* operon, it seems more likely that when the proper experiments are done, the *R. sphaeroides* puf promoter will resemble that of *R. capsulatus*.

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PHYSICAL MAPPING OF THE GENOME OF *RHODOBACTER CAPSULATUS*

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INTRODUCTION

Among the photosynthetic bacteria, *Rhodobacter capsulatus* has been particularly well characterized genetically (reviewed in Scolnik and Marrs, 1987). By a variety of fine structure genetic mapping techniques, it has been previously determined that most of the genes involved in the synthesis of the photosynthetic apparatus in this organism are clustered in a small region of the genome (Yen and Marrs, 1976), and that region has been cloned (Marrs, 1981; Taylor, *et al.*, 1983)) and some of the genes sequenced (Youvan, et al., 1984). Mapping by R plasmid-mediated conjugation has produced a circular linkage map of the chromosome (Willison, *et al.*, 1985). A detailed study of the overall organization of the chromosome of *R. capsulatus* should assist in the understanding of the global regulation of its genes, and the availability in cloned form of all parts of the genome will facilitate the identification and sequencing of additional genes. Towards these ends we are determining a physical map of the chromosome of *R. capsulatus*.

A physical genome map consists of an ordered set of DNA clones, and it may be constructed by finding the overlaps among a random library of genomic clones. A method for determining these overlaps by restriction fingerprinting has been developed and used in mapping the genome of the nematode, *Caenorhabditis elegans* (Coulson, *et al.*, 1986). An additional type of physical mapping consists of ordering the large chromosomal fragments that may be produced by the action of restriction enzymes at rare sites and separated by pulsed field gel electrophoresis. It has been shown previously that an *Xba*I digest of *R. capsulatus* DNA yields a small number of fragments (McClelland, *et al.*, 1987). We have used the restriction fingerprinting technique in conjunction with the ordering of large *Xba*I fragments to develop a physical genome map for *R. capsulatus*.

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