Molecular Genetics of Photosynthetic Membrane Biosynthesis in *Rhodobacter sphaeroides* 

PATRICIA J. KILEY* AND SAMUEL KAPLAN* 

Department of Microbiology, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801 

INTRODUCTION 

In the last 30 to 40 years, the study of photosynthetic membranes from the anoxicogenic and oxygenic photosynthetic procaryotes has provided a wealth of information pertaining to their biosynthesis and structure-function relationships of the various integral membrane, pigment-protein complexes. In particular, photosynthetic membranes derived from the gram-negative, purple nonsulfur, photoheterotrophic bacterium *Rhodopseudomonas sphaeroides*, recently renamed *Rhodobacter sphaeroides* (59), have been extensively characterized with regard to primary photochemistry, redox chemistry, bioenergetics, and structure, as well as in studies which pertain to the assembly of the photosynthetic membrane and its components (for reviews, see references 32, 41, 48, 63-65, 87, and 98). The aim of this review is to relate very recent advances in this field to previous work, so that we may begin to conceptualize, at the molecular level, how numerous, seemingly diverse synthetic pathways interact and how these pathways are regulated to ultimately allow the orderly assembly of this biological membrane system in response to a variety of environmental conditions. We focus our discussion on the photosynthetic membranes of *R. sphaeroides* since, in many respects, this represents the most thoroughly studied system to date and, when appropriate, we also compare and contrast these processes in other well-studied members of the *Rhodospirillaceae*; several recent reviews discuss specific aspects of this topic in other purple nonsulfur bacteria in more detail (43, 44, 99a, 136). 

*R. sphaeroides*, as well as other representatives of the purple nonsulfur photosynthetic bacteria, are capable of growth by aerobic and anaerobic respiration, fermentation, and anoxicogenic photosynthesis. In some members of this group the ability to fix atmospheric nitrogen permits an integrated study of these diverse metabolic activities often separated in other biologic systems. When growing chemoheterotrophically, *R. sphaeroides* has a typical gram-negative cell envelope and growth is supported by aerobic respiration. When oxygen is removed from such a culture, a series of events is triggered which results in the differentiation of the cytoplasmic membrane (CM) through a process of invagination into specialized domains which comprise the photosynthetic intracytoplasmic membrane system (ICM). The ICM is physically continuous with the CM but structurally and functionally distinct in that the ICM specifically contains all of the membrane components required for the light reactions of photosynthesis. Therefore, *R. sphaeroides* provides an excellent model system with which to study both photosynthesis and membrane development. An advantage to studying membrane biosynthesis in *R. sphaeroides* and other closely related facultative photoheterotrophic bacteria is the ability of these bacteria to synthesize photosynthetic membranes in the absence of light and under conditions in which these membranes are otherwise gratuitous for cell growth. For example, the photosynthetic membrane is synthesized when cells are growing chemoheterotrophically under low-oxygen partial pressures, using O₂ as a terminal electron acceptor, or under conditions of anaerobic respiration in the dark, using dimethyl sulfoxide (131), trimethyl-
amine-N-oxide (99), or other terminal electron acceptors. The mechanism by which reduced oxygen tensions or anaerobiosis translates into a biological signal to trigger induction of the ICM is unknown but may be mediated, as previously suggested, through a redox carrier(s) (29), repressor or activator protein(s), intracellular levels of a small molecule(s), or some combination of these effectors. Regardless of the signal(s), the gratuitous induction of ICM synthesis under defined physiological conditions allows analysis of ICM formation in both wild-type strains and those containing mutations in structural or regulatory loci which render them unable to grow photosynthetically. Moreover, in addition to regulation of ICM synthesis by oxygen tension, the molecular composition and intracellular amount of the ICM are regulated by incident light intensity. The amount of ICM per cell and the whole-cell specific bacteriochlorophyll \( a \) (Bchl) content increase proportionally as a result of decreasing the incident light intensity used for growth (Table 1; see Fig. 8). Regulation by cultural incident light intensities is also characterized by the differential synthesis of individual pigment-protein complexes (see subsection, “Induction and Assembly of the ICM”). Therefore, the inducibility of ICM synthesis by low-oxygen tensions has allowed study of the de novo synthesis of a functional biological membrane: by further varying conditions of incident light intensity, questions pertaining to how ICM synthesis and composition are physiologically regulated can also be addressed. As described in this review, de novo synthesis of the ICM refers to the morphologically distinguishable developmental process of differentiation of the CM leading to what we term mature ICM (see subsection, “Induction and Assembly of ICM”). In contrast, derepression in ICM synthesis can also occur as a result of the insertion of pigment-protein complexes into preexisting mature ICM which reveals no apparent morphological developmental distinction.

**ICM STRUCTURE AND FUNCTION**

When examined by electron microscopy, the ICM appears as vesicular invaginations budding from the cytoplasmic membrane (90). Upon cell disruption, the ICM fractions and comminutes to form numerous 50- to 60-nm-diameter spherical vesicles which are inside-out relative to the CM (52). The ICM vesicles, termed chromatophores, are photochemically active and can be easily purified from other cellular components (52). The ability to study the kinetics of light-induced electron transfer reactions in chromatophores has been central to elucidating the mechanisms of both the primary reactions of photosynthesis and cyclic photosynthetic electron transport in *R. sphaeroides* (31, 32). For these reasons, the best-studied functional ICM components are those involved in light energy capture and subsequent redox reactions, while parallel studies have followed the synthesis of these components in the ICM. It is not our intent to review the wealth of biophysical and spectroscopic studies pertaining to chromatophore function but rather to summarize major findings that relate to ICM synthesis and structure.

The most abundant chromatophore protein complexes are the Bchl- and carotenoid-containing light-harvesting (LH) complexes that have been designated B875 (formerly LHI) and B800-850 (formerly LHII), based on their near-Bchl infrared absorption maxima (25). The composition, function, and organization of LH complexes in the photosynthetic membranes from several photosynthetic bacteria have recently been reviewed (141, 142). As their name implies, the LH complexes act as antenna to funnel photons to reaction center (RC) Bchl-protein complexes in which light energy is converted to chemical energy by photo-induced oxidation-reduction reactions (98). The funneling of photons to the RC occurs by a process of exciton transfer, rather than light energy emission and backscatter (98). The arrangement of these Bchl-protein complexes in the ICM phospholipid bilayer must be highly organized to achieve the high efficiency of exciton transfer, since little light is emitted as fluorescence from wild-type strains during active photosynthetic growth (82). In photosynthetic bacteria such as *R. sphaeroides*, which contain both B875 and B800-B850 LH complexes, the B875 complex appears to be an obligatory intermediate in exciton energy transfer from the B800-850 complex to the RC complex (76). Aggregates of B875 complexes surround and possibly interconnect RC complexes within the ICM; these aggregates have been termed the fixed photosynthetic units since the ratio of B875/RC complexes is invariant at approximately 15:1 (1). Direct evidence for a symmetrical arrangement of LH complexes surrounding an RC has been demonstrated only in the photosynthetic bacterium *Rhodopseudomonas viridis* by immunoelectron microscopy, combined with image enhancement (109). Extrapolation of biophysical measurements suggest a similar arrangement in other purple nonsulfur bacteria (44, 82). The B800-850 complexes are peripherally associated around the fixed photosynthetic unit, and the amount of B800-850 complexes...

### Table 1. Effect of light intensity on ICM synthesis

| Light intensity (W/m²) | Generation time (h) | Specific Bchl (µg/mg cell protein) | Amt of ICM components in crude membranes | Relative amt of ICM polypeptides (densitometer units) | ICM vesicles/µm² cell membrane |
|------------------------|---------------------|----------------------------------|----------------------------------------|------------------------------------------------------|-----------------------------|
|                        |                     |                                  | B875⁺| B800-850⁺| RC-H⁺| RC-H⁺ | RC-M⁺ | RC-L⁺ | B875⁺⁺ | B800-850⁺⁺ | B875⁻⁻ | B800-850⁻⁻ | RC-H⁻⁻ | RC-H⁻⁻ |
| 3                      | 10.8                | 9.1                              | 13.7 | 18.7    | 2.2   |        |        |        |        | 1.751   | 2.057   | 1.348   | 21.5  | 4.2  |
| 10                     | 3.0                 | 4.6                              | 7.4  | 9.3     | 1.4   |        |        |        |        | 1.681   | 1.687   | 1.202   | 11.10 | 2.5  |
| 100                    | 3.0                 | 2.9                              | 7.4  | 4.0     | 1.0   |        |        |        |        | 1.646   | 731     | 1.290   | 6.47  | 1.9  |

* Crude membranes were prepared as described previously (40).
* Determined from [²⁵¹H]tyrosine-labeled chromatophores (67). Densitometer scans of X-ray films of sodium dodecyl sulfate-polyacrylamide gel electrophoresis resolved ICM isolated from *R. sphaeroides* 2.4.1 grown at different light intensities. The densitometer units were normalized for the moles of tyrosine per polypeptide.
* Cell membrane lengths (in centimeters) were measured on ×100,000 or ×150,000 enlargements, using a manual measuring wheel (for ×100,000, 1 cm = 0.1 µm; for ×150,000, 1 cm = 0.067 µm). The results were obtained from measurements of thin sections such as those shown in Fig. 8 (kindly provided by A. Varga).
* Determined from the extinction coefficient *E*ₙₐ₅₆₂₀ ≈ 73 M⁻¹ cm⁻¹ for the B875 complex normalized for 2 mol of Bchl *a* per complex. Similarly, the amount of B800-850 Bchl was calculated by the *A*ₙ₉₄₀₉₀ using *E* = 96 M⁻¹ cm⁻¹ (76), normalized for 3 mol of Bchl *a* expressed as nanomoles of spectral per milligram of crude membrane protein.
* Determined from a Western blot (immunoblot) analysis using anti-RC-H antibody, and quantitated by densitometer scans of X-ray films as described previously (40).
complex present in the chromatophore varies inversely with incident light intensity (1).

The absorption of light energy by the RC ultimately results in the photooxidation of a "special pair" of Bchl molecules (46, 85). Then, following a series of intermediate states, a quinone is reduced, diffuses from the Qb binding site of the RC complex, and equilibrates with the membrane-soluble quinone pool, thereby carrying an electron to the ubiquinol cytochrome c₃ oxidoreductase (cyt b/c₁) complex (31, 32). Cytochrome c₃ (cyt c₃) functions as a mobile periplasmic redox carrier in cyclic photosynthetic electron flow by transferring an electron from the membrane-bound cyt b/c₁ complex to the photooxidized RC complex (94). The kinetic parameters of this cyclic photosynthetic electron flow and the relative topology and distribution of individual redox centers within the complexes in the ICM bilayer are best understood in R. sphaeroides (31, 32), and an illustration depicting these components is shown in Fig. 1.

COMPOSITION OF ICM COMPLEXES

Several of the supramolecular complexes of the ICM have been purified to homogeneity, using detergent solubilization techniques. The analysis of purified complexes has been central to determining the minimal subunits required for, as well as the structural basis of, their functional activity. The B800-850 complex has been purified following lauryl dimethylamine-N-oxide detergent solubilization of chromatophore membranes (24). The minimal unit required for B800-850 spectral activity appears to consist of six molecules of Bchl, three molecules of carotenoid, and two each of two small hydrophobic polypeptides designated B800-850-α and B800-850-β (R. Cogdell, personal communication; 24, 117). Unlike Rhodobacter capsulatus, there is no 14,000-dalton polypeptide (45) associated with the purified R. sphaeroides B800-850 complex. The primary amino acid sequence and cognate deoxyribonucleic acid (DNA) sequences of B800-850-α and B800-850-β have been described previously, and the sizes of these polypeptides have been determined to be 5,599 and 5,448 daltons, respectively (116, 117). Previously, Cohen and Kaplan purified and characterized three low-molecular-weight polypeptides from photosynthetic membranes, designated 15A, 15B, and 15C, which were thought to be components of the LH machinery due to their abundance in purified chromatophores (27, 28). The polypeptide designated 15A does not appear to be a component of any of the purified spectral complexes. In addition, the 15A polypeptide cannot be detected immunologically in a mutant deficient in the B800-850 complex (67a). It is possible that polypeptide 15A has a functional role associated with assembly or synthesis of the B800-850 complex and may be analogous to the 14-kilodalton polypeptide (45) that can be isolated with the R. capsulatus B800-850 complex. Analyses have shown that antisera against 15B and 15C cross-react with the B800-850-β polypeptide (M. Morneau, senior thesis, University of Illinois, Urbana, 1984). Thiefer et al. (116, 117) have shown from amino acid sequence analysis that there is heterogeneity at the amino-terminal end of the R. sphaeroides B800-850-β polypeptide; some polypeptides contain amino-terminal methionine, and the remainder had a blocked amino-terminal threonine. It is possible that the difference in charge between 15B and 15C may relate to the heterogeneity at the N terminus of the B800-850-β polypeptide observed by Thiefer et al. (116, 117) and that this could be the result of posttranslational modification of the B800-850-β polypeptide.

The purified B875 complex contains the B875-β and B875-α apoproteins in a 1:1 stoichiometry, per two molecules each of Bchl and carotenoids (10). Both B875-β and B875-α apoproteins have been sequenced, and their molecular weights have been determined to be 5,457 and 6,809, respectively (115). A similar analysis of the LH complexes from several other photosynthetic bacteria has been performed (141, 142).

Secondary structure predictions of the α and β subunits from both LH complexes show overall similarities (Fig. 2). The LH polypeptides contain a single conserved histidine residue which may function in Bchl binding and a central hydrophobic domain predicted to span the ICM bilayer once. The conserved histidine residues presumed to be the axial ligands to the central magnesium atom of Bchl are located within this hydrophobic domain. The B800-850-β polypeptides from both R. capsulatus and R. sphaeroides contain an additional histidine residue which is also located in the hydrophobic domain and may also bind Bchl.

FIG. 1. Illustration of a portion of the ICM bilayer depicting the relative orientation of specific ICM components. DH, Dehydrogenase: NADH, reduced nicotinamide adenine dinucleotide; ADP, adenosine 5'-diphosphate; ATP, adenosine 5'-triphosphate.
(141-143). Studies of the iodination and protease sensitivities of chromatophores of *Rhodobacter capsulatus* (92, 110) or *Rhodospirillum rubrum* (13) show that the LH polypeptides are oriented in the bilayer such that the amino terminus protrudes into the cytoplasm whereas the carboxy end is located in the periplasmic space. A model of the *Rhodospirillum rubrum* B880 complex (142, 143) that demonstrates this orientation is shown in Fig. 3. Although recent attempts to crystallize LH complexes have been successful (120), the quality of the crystals has not yet been suitable for high-resolution X-ray diffraction studies. The three-dimensional structure of the LH complexes is required to precisely determine the location of Bchl molecules and the organization of the pigments and polypeptides within these complexes.

The RC complex from *R. sphaeroides* contains three polypeptides, RC-H, RC-M, and RC-L (based on their relative mobilities from sodium dodecyl sulfate-polyacrylamide gel electrophoresis of 28,000, 24,000, and 21,000 daltons, respectively), in a 1:1:1 stoichiometric ratio with 4 molecules of Bchl, 2 molecules of bacteriopheophytin, 2 molecules of ubiquinone, a nonheme iron, and a single carotenoid (48). Calculations of the subunit molecular weights from the deduced primary sequence of the *R. sphaeroides* genes reveal that the RC-M (34,265 daltons) and RC-L (31,319 daltons) subunits are actually larger than the RC-H subunit (28,003 daltons). The estimated molecular weight for the whole *R. sphaeroides* RC complex including cofactors is 100,858 (122).

The RC complexes from both *Rhodopseudomonas viridis* and *R. sphaeroides* have been crystallized. The structure of the RC from *Rhodopseudomonas viridis* has been determined to a resolution of ~0.25 nm (38, 78), whereas that of *R. sphaeroides* has been determined to ~0.28 nm (3, 4, 128). Although the crystallized RC complex from *Rhodopseudomonas viridis* also contains 1 mol of a membrane-bound c type cytochrome (38, 78, 121), the major structural features of the RC from both bacteria have been conserved, and the structure of the chromophores from the *R. sphaeroides* RC is shown in Fig. 4. The RC-L and -M subunits are both integral membrane polypeptides which are in intimate association with the chromophores shown in Fig. 4. The third subunit, RC-H, has a single, amino-terminal, membrane-spanning region, but the majority of the protein is hydrophilic and appears to protrude into the cytoplasm. Since it is known that the RC-H polypeptide does not bind any cofac-
tors and is not required for some aspects of RC function in vitro (2), there has been much speculation as to its role. For example, it has been determined that substantial amounts of RC-H can be found in aerobically grown cells where there are no detectable RC-M or RC-L polypeptides (16, 40). Moreover, there is an approximately 50% excess of the RC-H polypeptide relative to RC-M and RC-L in the bulk photosynthetic membrane (P. J. Kiley, Ph.D. thesis, University of Illinois, Urbana-Champaign, 1987). Analysis of *R. sphaeroides* mutants deficient in the RC-H subunit and its role in RC structure or photosynthetic membrane assembly or both are discussed later. It might be inferred that the RC-H subunit is not necessary for RC function since some photosynthetic bacteria (i.e., *Rhodococcus gelatinosa*) contain RC complexes with only two subunits (21). When DNA probes from *R. sphaeroides* specific to the RC-H, -M, and -L genes were used against *Rhodococcus gelatinosa* genomic DNA, strong homologies to RC-L and RC-M were observed but the RC-H probe detected no signal, even at low stringencies of hybridization (W. Havelka and S. Kaplan, unpub-

FIG. 3. Structure model of the transmembrane-oriented LH polypeptides from purple photosynthetic bacteria (amino acid residues of the α- and β-polypeptides from *Rhodospirillum rubrum*). An α-helical conformation of the hydrophobic domain within the hydrocarbon tail region is assumed. The possible Bchl-binding site is at the His residues (one His residue in the α- and β-polypeptide each, near the periplasmic side of the membrane, exciton-coupled Bchl pair; single His residue in the β-polypeptide monomeric Bchl). PK, CH, SA, and TR are sites of partial hydrolysis with proteinase K, chymotrypsin, Staphylococcus aureus proteinase, and trypsin at the N-terminal domains of the α- and β-polypeptides located at the cytoplasmic side. Reprinted from Zuber (143) with permission of Springer-Verlag.

FIG. 4. Stereoplot of the cofactors of the RC from *R. sphaeroides* at a resolution of 0.33 nm. The twofold symmetry axis is aligned vertically in the plane of the paper, and the electron transfer proceeds preferentially along the A-branch. The inferred position of the membrane is indicated by dashed lines. Reprinted with permission of Allen et al. (4).
lished results). However, specific antibody to \( R. \) \textit{sphaeroides} RC-H showed a cross-reaction with the RC-L and RC-M polypeptides of \textit{Rhodococcus gelatinosa} but no unique signal for RC-H was detected in membrane fractions from \textit{Rhodococcus gelatinosa} (J. Hoger and S. Kaplan, unpublished results). Therefore, it may be proposed that in \textit{R. sphaeroides}, RC-H has functions that may be served in \textit{Rhodococcus gelatinosa} by (parts of) RC-L or RC-M or both.

Other protein complexes in the \textit{R. sphaeroides} ICM that have been characterized include the cyt \( b(c)_1 \) complex which consists of equimolar amounts of cyt \( c_1 \) (33 kilodaltons), a cyt \( b \) (40 kilodaltons) which contains two hemes, and a Rieske iron-sulfur protein (24 kilodaltons) (55). It has been estimated that chromatophores from cells grown at relatively high light intensities contain approximately 0.5 mol of the cyt \( b(c)_1 \) complex per mol of flash-oxidizable RC (31). The soluble periplasmic cyt \( c_2 \) is also present in the ratio of 0.5 mol/mol of RC (31). Cyt \( c_2 \) consists of a single subunit of 14,200 daltons with one covalently attached heme (77), although recent studies suggest the existence of multiple electrophoretic forms of this protein in the \textit{R. sphaeroides} periplasm (T. J. Donohue, A. E. McEwan, S. van Doren, A. R. Crofts, and S. Kaplan. Biochemistry, in press).

Previous studies have suggested the existence of multiple cyt \( c_2 \)-type species in other species of purple nonsulfur bacteria (46, 49), although the physiological significance of these observations is yet to be determined.

Until recently, it was assumed that the mechanism of cyclic electron transfer was the same in \textit{R. capsulatus} as it was in \textit{R. sphaeroides}. As expected from the previously mentioned pathway of cyclic photosynthetic electron flow, cyt \( c_2 \)-cyt \( c_1 \) mutants from \textit{R. sphaeroides} were incapable of growth by photosynthesis (Donohue et al., in press). The rate of RC reduction in \textit{cycA} mutant strains grown under conditions gratuitous for ICM synthesis was approximately 10,000-fold slower than in the wild-type strain (Donohue et al., in press). These results provided genetic support for the earlier spectroscopic studies which demonstrated that cyt \( c_2 \) was the immediate electron donor to the \textit{R. sphaeroides} RC (94). In contrast, \textit{cycA} mutants in \textit{R. capsulatus} were previously shown to be capable of photosynthetic growth (33); kinetic studies demonstrate a cyt \( c_2 \)-independent route of RC reduction which was not dependent on a periplasmic redox protein (95), since this process occurred in spheroplasts from both wild-type and \textit{cycA} mutants. Although this difference in photosynthetic electron transport between \textit{R. capsulatus} and \textit{R. sphaeroides} was initially unexpected, the results obtained are now seen as consistent with previous studies on the differential inhibition of light-induced electron flow by antibodies against cyt \( c_2 \) (94). The immediate electron donor to the \textit{Rhodopseudomonas viridis} RC is a membrane-bound cyt \( c_2 \) type cytochrome which has recently been shown to be a lipoprotein (121). Michel and co-workers (121) have proposed that the fatty acids function to anchor the cytochrome subunit in the photosynthetic membrane. Even though cyt \( c_2 \) is not the immediate electron donor to the RC in \textit{Rhodopseudomonas viridis}, a periplasmic cyt \( c_2 \) appears to stimulate RC reduction in vitro (103).

**GENETIC ORGANIZATION AND IDENTIFICATION OF THE STRUCTURAL GENES FOR ICM COMPONENTS**

**Chromosomal Mapping**

A classical genetic analysis of the \textit{R. sphaeroides} and \textit{R. capsulatus} genomes has utilized chromosome transfer techniques that use promiscuous R plasmids (75, 106, 108, 125). Miller and Kaplan were the first to show the usefulness of promiscuous R plasmids to the genetic analyses of photosynthetic bacteria (81). Since then, several groups have used these plasmids to develop genetic techniques in the photosynthetic bacteria (75, 106, 108, 125). Sistrom and co-workers demonstrated conjugal transfer of chromosomal genes in \textit{R. sphaeroides} mediated by the broad-host-range, self-mobilizable plasmid R68.45 (106) and, using a variety of auxotrophic and photosynthetic deficient mutants, ordered several alleles on the chromosome through a series of two- and three-factor crosses (Fig. 5). Similarly, Pemberton and co-workers (88, 108), using chromosome transfer techniques and linkage analyses, mapped six genes involved in carotenoid biosynthesis (\textit{crtA}, \textit{crtB}, \textit{crtC}, \textit{crtD}, \textit{crtE}, and \textit{crtF}) relative to the chromosomal genes \textit{phe-2}, and \textit{arg-4} (Fig. 6).

Using a replication temperature-sensitive R plasmid, pTH10, Willison et al. (125) generated a circular linkage map of the \textit{R. capsulatus} B10 chromosome which demonstrated that mutations affecting nitrogen fixation were dispersed in several linkage groups on the chromosome. Fine-structure mapping of several \textit{nif} mutations was accomplished by using the gene transfer agent specific for \textit{R. capsulatus} (125). Gene transfer agent acts similar to a generalized transducing phage in that it packages random fragments of chromosomal DNA (approximately 5 kilobases [kb] in length), but unlike a more typical transducing phage it cannot independently replicate (74). Willison et al. (125) further showed that the photosynthetic gene cluster maps between one of the \textit{nif} gene clusters and a gene for histidine biosynthesis. It is interesting that a \textit{nif} mutation isolated from \textit{R. sphaeroides} also maps near the genes for carotenoid and Bchl biosynthesis (88).

With the availability of the techniques of molecular genetics, more sophisticated techniques of chromosomal mapping are being developed. Dryden and Kaplan (unpublished results) as well as Weaver and Tabita (119) have constructed cosmid banks by using vector systems that can be stably maintained in \textit{R. sphaeroides}, and similar chromosomal banks have been constructed from \textit{R. capsulatus} (68). Since the average cosmid generated in these studies carries 25 to 35 kb of insert DNA, a minimum of 200 random isolates is sufficient to represent the entire chromosome assuming a genome size of approximately 5,000 kb. Therefore, these banks will be useful for chromosomal mapping studies as well as for the identification of genes by mutant complementation analysis. By the use of orthogonal field gel electrophoresis, the \textit{R. sphaeroides} chromosome can be resolved into a minimum of 13 DNA \textit{DraI} restriction enzyme frag-

![FIG. 5. Linear genetic linkage map of the \textit{R. sphaeroides} WS2 chromosome. Courtesy of A. Maculosa and W. R. Sistrom.](image-url)
ments ranging in size from approximately 50 to >600 kb (W. Y. Qiang and S. Kaplan, unpublished results). From this analysis, the genes encoding the following proteins were found to reside on a unique approximately 650-kb DraI fragment: cyt C2, phosphoribulokinase, fructose bisphosphate, ribulose 1,5-bisphosphate carboxylase/oxygenase (form II), 5-aminolevulinic acid (ALA) synthase, and B800-850, B875, and RC-H, RC-M, and RC-L polypeptides. Furthermore, the genes for cyt C2 and the B800-850, B875, and RC polypeptides all hybridized to an approximately 100-kb region of *R. sphaeroides* DNA from the *R. pWS2* (106, see subsection. “Identification of Photosynthetic Genes”). By use of the cloned genes described in this review together with a number of Tn5-induced auxotrophic markers, the linkage relationships of those DNA regions contributing to ICM structure, function, and synthesis can be determined. Recently, two strains of *R. sphaeroides* have been described that will aid in complementation analysis since, phenotypically, one appears to be devoid of the endogenous restriction enzyme system and the second mutant may be deficient in recombination activity (89, 106).

**Site-Specific Mutagenesis**

Generation of site-specific insertion mutations in the photosynthetic bacteria, designated interposon mutagenesis, was first demonstrated in *R. capsulatus* (135). In these studies, gene transfer agent was used as the vehicle to deliver genes that had been inactivated or replaced by antibiotic-resistant cartridges back to the *R. capsulatus* chromosome by homologous recombination (135). By using a slightly different approach, site-specific insertion mutations have been constructed in *R. sphaeroides* through the use of the suicide vector system of Simon et al. (104), which allows for a homologous recombination event to occur between the modified gene on an unstable plasmid, such as pSUP202, and the wild-type gene on the *R. sphaeroides* chromosome. Whereas the pBR replicon appears to be incapable of replicating in *R. sphaeroides* (50), it appears to function normally in *R. capsulatus* (135). Such site-specific mutations have been constructed in numerous photosynthetic genes with relatively high frequencies in both *R. capsulatus* (33, 35) and *R. sphaeroides* (37; Donohue et al., in press). The analysis of such mutants has been instrumental in and will continue to aid in our understanding of the mechanisms of photosynthetic electron transfer and the regulation, structure, and function of ICM components, as has been referred to at various points in this review.

**Utility of the lac Operon**

Nano et al. (85) were the first to demonstrate the potentiality of the *Escherichia coli lac* system for molecular genetic analyses in the photosynthetic bacteria by demonstrating the application of this system to *R. sphaeroides*. Since *R. sphaeroides* is naturally unable to use lactose as a carbon source, expression of the *E. coli lac* operon dependent on transcriptional or translational fusions of *R. sphaeroides* DNA to *lacZ* provides a very powerful genetic tool both to study the expression of specific genes and to select for mutations that affect expression of various gene fusions. Recently, T. Tai, W. Havelka, and S. Kaplan (submitted for publication) have prepared a variety of plasmid constructions based on the RSF1010 replicon that facilitate cloning and the construction of translational *lacZ* fusions in *R. sphaeroides*.

**Gene Expression**

The recent advances in molecular genetics in the photosynthetic bacteria has led to studies of gene expression for those ICM components described above. Numerous studies have noted the general lack of expression of *R. sphaeroides* genes in *E. coli* (17, 19, 83, 84). The difference in expression appeared to be at the transcriptional level, which may stem from the difference in the genomic G+C content of DNA from *R. sphaeroides* (68 to 70 mol% G+C; 93) and that of *E. coli* (50 to 51 mol%), although other possibilities have not been ruled out. To our knowledge, no *E. coli* ~10~ and ~35~ consensus promoter sequences have been found upstream of any genes so far sequenced from any species of photosynthetic bacteria.

To study gene expression in these bacteria, two in vitro systems have been developed. A homologous coupled transcription-translation system from *R. sphaeroides* has been used to express *R. sphaeroides* genes from exogenously added DNA templates (17, 19, 40, 42, 57, 66, 67). This same system has proven to be of general utility in its ability to express DNA sequences from other high-G+C procaryotes (J. Chory, T. Donohue, P. J. Kiley, W. Havelka, and S. Kaplan, unpublished observations). Ribonucleic acid (RNA) polymerase from both *R. capsulatus* (51) and *R. sphaeroides* (J. Kansy and S. Kaplan, manuscript in preparation) have been purified, and an in vitro transcription assay system is currently being used to identify promoter regions for various photosynthetic genes. Although 5' ends of several stable messenger RNA (mRNA) species have been mapped in several species of photosynthetic bacteria (7, 56, 118, 140) and attempts have been made to compare potential upstream
regulatory sequences, it is our feeling that sufficient data are not available at this time to unambiguously identify canonical promoter sequences in any of the photosynthetic bacteria.

**Identification of Photosynthetic Genes**

From genetic studies, it has been shown that the genes encoding the Bchl and carotenoid biosynthetic enzymes, and the subunits for the B875 and RC pigment-protein complexes, are clustered in both *R. sphaeroides* (88, 106) and *R. capsulatus* (75, 114, 130). This was first demonstrated by the isolation of an R-prime from *R. capsulatus* carrying an approximately 50-kb insert of *R. capsulatus* DNA (pRPS404) which could complement most mutants deficient in various aspects of photosynthetic function (75). It was later shown that this plasmid contained the genes for the latter steps in carotenoid biosynthesis (the enzymes required to synthesize the colored carotenoid species from the ultraviolet light-absorbing phytoene: 100). Bchl biosynthesis (the enzymes that convert protoporphyrin IX to Bchl), and the structural genes for the RC and B875 polypeptides (114, 132). Sistrom et al. have subsequently isolated an R-prime, pWS2, from *R. sphaeroides* which appears to be functionally equivalent to the *R. capsulatus* R-prime pRPS404 (106). Pemberton and Harding (88) constructed a cosmid bank from *R. sphaeroides* and by complementation analysis have identified cosmids carrying six different genes involved in carotenoid biosynthesis. Recently, two separate structural genes (hemA and hemT) for ALA synthase have been cloned from *R. sphaeroides* (T. Tai and S. Kaplan, manuscript in preparation), using a heterologous probe containing the hemA gene of *Rhizobium mellioti* (72). ALA synthase is responsible for the synthesis of ALA, which is the first committed precursor in tetrapyrrole biosynthesis. Neither of these genes are close to the *puf* or *pufM* operons of *R. sphaeroides* as determined by restriction enzyme mapping (see “Chromosomal Mapping”). Another key biosynthetic pathway necessary for ICM formation is isopenidren synthesis since it is a precursor of carotenoids, phytol (for Bchl synthesis), and isopenidren quinones. The key regulatory enzyme in isopenidren biosynthesis in eucaryotes is the 3-hydroxy-3-methylglutaryl coenzyme A reductase. When Dryden and Kaplan (unpublished results) attempted to identify this gene in *R. sphaeroides*, using synthetic deoxyoligonucleotides based on the conserved region within the catalytic domain of 3-hydroxy-3-methylglutaryl coenzyme A reductase as probes in a Southern hybridization analysis, three unique EcoRI restriction fragments were identified. Each individual fragment was cloned and subsequently shown to contain a common homologous *PvuII* restriction fragment which also contained homology to the synthetic probes. It is not known at this time whether this represents three distinct genes for this enzyme and, if this is the case, whether all three genes are functional and, if so, the nature of their physiological roles.

Several of the structural genes for previously described ICM components have been cloned by using either heterologous gene probes or synthetic deoxyoligonucleotides designed from the available cognate amino acid sequences. In general, most of the genes encoding subunits of a particular complex are linked in *R. sphaeroides* (5, 40, 66, 67, 122–124), *R. capsulatus* (132, 134), *Rhodopseudomonas rubrum* (88), and *Rhodopseudomonas viridis* (79, 80), with one very notable exception. In those bacteria that have been examined, the structural genes for two of the RC polypeptides, RC-L and RC-M, designated *pufL* and *pufM*, respectively, are transcriptionally linked to the two structural genes for the α and β subunits of the B875 complex, *pufA* and *pufB*, respectively (7, 139, 140). The structural gene for the third polypeptide in the RC, RC-H (designated *pufA*), is not linked to the *puf* operon, and preliminary data suggest that it maps at least 40 kb away in *R. sphaeroides*, as it does in *R. capsulatus* (114). The structural genes for the B800-850 subunits α and β are also linked to one another and comprise the *puc* operon (5, 67, 134).

The genes encoding the subunits of the cyt b6f complex have recently been cloned in both *R. capsulatus* (35, 36) and *R. sphaeroides* (56) and comprise an operon. The gene order is Reiske iron-sulfur protein, cyt b, and cyt c1, or *fbc* as first designated by Gabellini et al. (56). The original identification and cloning of these genes were reported to be from *R. sphaeroides* (56), although it was subsequently shown that the source of these genes was most likely *R. capsulatus* (36). This apparent discrepancy originated from the use of a supposed *R. sphaeroides* mutant strain, GA, that was later shown to have been most probably derived from *R. capsulatus* (36). This green mutant GA used by Gabellini et al. should not be confused with the *R. sphaeroides* 2.4.1 derivative Ga, isolated by Cohen-Bazire (29, 105), which we have recently reconfirmed is from 2.4.1 by phage typing (T. Donohue and S. Kaplan, unpublished results). Subsequently, the *fbc* operon has been reisolated and characterized from both species (36). The gene encoding the soluble cyt c2 (cyt c1) has also been cloned, and its map location is known in *R. sphaeroides* (see “Chromosomal Mapping”). The membrane-bound cyt c from *R. viridis* has been identified downstream of the genes for the RC-L and RC-M polypeptides (79). Presumably, it is cotranscribed with the *R. viridis* *puf* operon, since the initiation codon for the cyt c gene overlaps with the termination codon for *pufM* (79). This difference in genetic organization between the *puf* operon of *R. viridis* and that of *R. sphaeroides* and *R. capsulatus* is interesting in light of the differences in the three-dimensional crystal structure of the RC (see “Composition of ICM Complexes”). In both *R. sphaeroides* (B. Dehoff and S. Kaplan, unpublished results) and *R. capsulatus* (132), there is a different open reading frame downstream of *pufM* that would code for a hydrophobic 82-amino-acid polypeptide (*pufX*). This segment of DNA is cotranscribed with the large *puf* operon transcript (see section entitled Expression of the *puf* operon) in both *R. sphaeroides* (R. Gyure and T. Donohue, personal communication) and *R. capsulatus* (138), although the function of this polypeptide is presently unknown. There is no information currently available as to the existence of a similar open reading frame downstream of the gene for the membrane-bound cyt c of the *R. viridis* RC. It is possible that the *pufX* gene product could play a role in RC structure or assembly together with B875 complexes.

**REGULATION OF ICM SYNTHESIS**

When considering the biosynthesis and regulation of ICM formation in *R. sphaeroides*, it is preferable to analyze steady-state growing cell cultures. Light intensities of 10 W/m² (measured through >650-nm cutoff filters) have been used for many physiological experiments involving photosynthetic cells, and cells exposed to a 25% O₂ atmosphere (this represents 100% dissolved O₂ in the culture medium; 16) have been used for aerobic chemoheterotrophic growth. At 10-W/m² light intensity, *R. sphaeroides* 2.4.1 has an
optimum growth rate of approximately 2.5 to 3.0 h but still derepresses both the amount of intracellular ICM and B800-850 complexes relative to cells grown at supersaturating light intensities such as 100 W/m² (18). One major problem that has arisen in studying the effects of changes in oxygen concentration or light intensity on various aspects of ICM biosynthesis is the variability in cultural conditions used in independent laboratories. Therefore, it has been difficult to compare the magnitude of the effects observed from study to study, and thus one can only consider the relative changes. For example, total repression of ICM synthesis in *R. sphaeroides* during active growth requires high oxygen tensions (16); cells grown at high aeration on a gyratory shaker (commonly used laboratory conditions) quite often do not generate high enough cultural oxygen tensions to repress ICM synthesis even at low cell densities. Only a few studies have actually characterized the levels of various photosynthetic related activities in cells grown in high oxygen tensions (16, 40, 42, 67, 138, 139), and therefore we know the basal level for only a relatively few activities in cells that are completely repressed for ICM synthesis. Superimposed on these difficulties is the tendency to directly compare or equate what are apparently complex physiological responses to changes in oxygen tension or light intensity in different species of photosynthetic bacteria, which may or may not be valid.

Previous studies have described the requirement for the coordinate synthesis of Bchl and protein for assembly of the ICM (11, 12, 113). In contrast, concurrent insertion of phospholipids (102) or carotenoids (29, 34) into the ICM of steady-state cultures is not required for the assembly of functional pigment-protein complexes. One possible exception to this is that assembly of B800-850 complexes may be tightly coupled to synthesis of colored carotenoids since all simple mutants described to date that lack colored carotenoids do not synthesize wild-type B800-850 complexes (5, 34, 84, 105). The coupling between ICM protein and Bchl synthesis has been best described in synchronous cultures of *R. sphaeroides* (65) in which the ICM phospholipid/protein ratio varies throughout the cell cycle due to the continuous insertion of protein into the membrane, with the movement of previously synthesized phospholipid into the ICM occurring just prior to cell division. In asynchronous steady-state photosynthetic cells, the average ICM protein/phospholipid ratio in the population remains constant, and this ratio is independent of the light intensity for growth (129). However, increases in the amount of ICM per cell in response to changes in light intensity are accompanied by increases in the cellular amount of phospholipid and Bchl-protein complexes (18, 65).

**Bchl Synthesis**

Coupling between synthesis of a polypeptide and its ligand (i.e., Bchl), as in the assembly of the LH and RC complexes, is an integral part of assembling the photosynthetic membrane. This is supported by the fact that wild-type strains do not have detectable pools of free Bchl, LH, or RC polypeptides and that mutants blocked in Bchl synthesis do not synthesize ICM or accumulate large quantities of Bchl-binding proteins, even under grape juice growth conditions for ICM synthesis (see above: 11, 12, 70, 113). The regulation of Bchl synthesis in *R. sphaeroides* is complex, since intermediates in the early part of the biosynthetic pathway are used under photosynthetic conditions for synthesis of two other tetrapyrrole-containing compounds, namely, hemes and carotenoids (71, 96). Moreover, the ability to synthesize Bchl under photosynthetic conditions without interrupting the flow of intermediates to heme or carotenoids is further evidence of the complexity of this pathway since the Bchl/heme ratio is 10 to 50:1 in photosynthetic cells (71, 96). Further compounding this regulatory problem is the absence of information on how bacteriopheophytin (Mg-free derivative of Bchl, found only in the RC complex) is synthesized.

Analysis of individual steps specific to the Bchl branch of this pathway has been hampered by the difficulty of assaying and purifying the enzymes involved in Bchl synthesis. Consequently, most of the steps in Bchl biosynthesis have been defined by the characterization of mutants blocked at various steps in the pathway. This subject has been extensively reviewed by Lascelles and co-workers (71, 96) and Jones (62). The complementation experiments of Marrs and co-workers (75, 130, 133) identified the genes required for synthesis of Bchl from *R. capsulatus* which were localized on the R-prime pRPS404, and a physical-genetic map was subsequently generated for this cluster of genes (114). To this date, the activities encoded by the cloned Bchl genetic loci have not been demonstrated, nor has the DNA sequence for these proposed genes been determined in any of the photosynthetic bacteria; neither of these tasks (especially the former) will prove to be easy. Confirmation of the proposed steps in the Bchl biosynthetic pathway awaits the demonstration of these activities in vitro.

Since the final product, Bchl, is relatively easy to assay, most of what is known about the regulation of Bchl biosynthesis in *R. sphaeroides* is known from examining cellular levels of this end product. For example, chemoheterotrophically grown cells of *R. sphaeroides* in high oxygen atmosphere show no detectable Bchl or ICM invaginations (16). Reduction or removal of oxygen from chemoheterotrophically growing cells induces synthesis of both Bchl and the ICM. In *R. capsulatus*, studies attempting to describe the transcriptional regulation of the Bchl biosynthetic genes have shown that hybridization to two BamHI restriction fragments containing the *bchJ*, *G*, *D*, *H*, and *F* genes of the Bchl branch of the biosynthetic pathway could be observed with mRNA isolated from aerobically grown cells of *R. capsulatus* (20, 138). It is not clear whether these cells were fully repressed for ICM synthesis. Biel and Marrs (9), using transcriptional *lacZ* fusions into *bchB, bchC, bchG*, and *bchH*, showed a twofold induction of β-galactosidase expression when *R. capsulatus* was grown under low aeration versus high aeration, which was similar to the results obtained through RNA hybridization studies with specific restriction fragment probes (20, 138). However, since the delineated boundaries of these genetic loci can represent only a minimal estimate of both gene size and number, one has not yet been able to correlate the levels of mRNA with any specific enzyme activity, especially since the restriction fragments used in these studies were not specific to one genetic locus (20, 138) and sufficient information is not available on the number or transcriptional organization of individual structural genes within these loci.

Several steps have been implicated as being important to the control of Bchl synthesis: (i) the activity of ALA synthase (ALA is the first committed precursor in the tetrapyrrole pathway); (ii) the branch point where either iron (heme biosynthesis) or magnesium (Bchl biosynthesis) is incorporated into protoporphyrin IX; (iii) the synthesis of phylitol side chain of Bchl; (iv) synthesis of a carrier for Bchl.
intermediates as proposed initially by Lascelles and co-workers (71, 96) and more recently by Bauer and Marrs (C. E. Bauer and B. L. Marrs, Abstr. Mol. Biol. Photosynth. Procaracteria, 1987, p. 21). Early studies by Lascelles and co-workers demonstrated that synthesis of the ALA synthase and S-adenosylmethionine-Mg-protoporphyrin methyltransferase (encoded by bchH) were repressed by growth of *R. sphaeroides* under high aeration on a gyroratory shaker (71). S-Adenosylmethionine-Mg-protoporphyrin methyltransferase is the only enzyme specific to the Bchl branch for which an enzyme activity has been demonstrated in vitro, and from these studies the incorporation of Mg into protoporphyrin IX appears to be coupled to the methylation step (96). Therefore, it has not been possible to compare enzymatically the Mg or ferrochelatase activity in terms of O$_2$ sensitivity, inducibility by anaerobiosis, or their relative affinities for protoporphyrin IX. Genetic evidence for the existence of a Bchl carrier has been recently suggested in *R. capsulatus* (the proposed function of the *Q* gene described by Bauer and Marrs) (see "Expression of the *out* Operon"). Although the function and characterization of *Q* gene product has not yet been demonstrated in vivo, mRNA specific to this gene has only been detected under photosynthetic conditions in *R. sphaeroides* (J. Lee and S. Kaplan, unpublished results).

Photosynthetically grown cells have been shown to contain more ALA synthase activity than chemoheterotrophically grown cells (71). Two forms of ALA synthase have been demonstrated in *R. sphaeroides* and the second form may be membrane associated (47). One way of sequestering intermediates for Bchl synthesis could be by compartmentalization of these at the ICM where Bchl will ultimately be inserted as components of the Bchl-protein complexes. The putative Bchl carrier protein mentioned above might function in this role as would the suggested membrane association of ALA synthase. ALA synthase requires a reducing environment for enzyme activity, and in vitro ALA synthase can be activated by a thioredoxin/thioredoxin reductase system (22, 23). It has been postulated that the levels of thioredoxin in vivo may be related to the redox state of the cell and, therefore, this protein may play a role in light regulation of Bchl synthesis in photosynthetic bacteria as has been previously suggested for plants (22, 23, 96). In this regard, since light regulation of Bchl synthesis has been suggested to be controlled by the available pool of ALA (86), the amount of reduced thioredoxin in photosynthetic cells may also be influenced by light-induced electron flux under photosynthetic conditions.

Recently, Tai and Kaplan (in preparation) have identified two unlinked restriction endonuclease fragments that hybridize to a *Rhizobium meliloti* structural gene (*hemA*) for ALA synthase. Each of these DNA fragments complements both *E. coli* and *R. sphaeroides hemA* mutants, and for one isolate high levels of ALA synthase activity were detected in the complemented strain. Therefore, it is interesting to speculate that these may represent two copies of the *hemA* gene and that each gene may be regulated differentially with respect to oxygen or light or both. Thus, we would suggest that mutants requiring ALA specifically for photosynthetic growth should be unobtainable because loss of membrane-bound ALA synthetase activity would be compensated for by the freely available pool of ALA produced by the soluble ALA synthase. Clearly, elucidating the details of the molecular mechanisms controlling Bchl synthesis is imperative to understanding not only Bchl synthesis but also how the photosynthetic membranes interact with other molecules to regulate assembly of the photosynthetic membrane. The identification and analysis of the genes and gene products for enzymes in the Bchl (9, 30, 130) and carotenoid (88, 100, 130) biosynthetic pathways in *R. capsulatus* and *R. sphaeroides* will thus be instrumental to eventually understanding the coordinate regulation of these complex biosynthetic pathways.

**ICM Assembly in Synchronous Cell Populations**

Under steady-state photosynthetic growth conditions, a cell cycle-dependent synthesis of the ICM has been observed. This topic has been extensively reviewed (65) and is only briefly summarized here. Following cell division, LH and RC complexes and other membrane components (e.g., adenosine triphosphatase), as well as many unidentified proteins, are continuously incorporated into preexisting ICM (53, 58, 127). Whole-cell phospholipid synthesis, although transiently interrupted at the time of cell division, does not result in new phospholipid being incorporated into ICM during the course of the division cycle (14, 73). Immediately preceding, or at the time of cell division, previously synthesized phospholipids are mobilized and incorporated into the replicating ICM as it is being partitioned to daughter cells.

The consequences of this cell cycle-specific uncoupling of protein and lipid insertion into the ICM are manifold and well documented, namely: (i) the ICM protein/phospholipid ratio fluctuates in a cyclical pattern throughout the cell cycle (54); (ii) the intrinsic density of the ICM fluctuates in a similar cyclical fashion (54); (iii) the fluidity of the ICM phospholipid bilayer reveals a cyclical pattern (65); (iv) the intramembranous particle distance between photosynthetic units changes directly with the protein/phospholipid ratio (129); (v) the movement of electrons through the electron transport chain reveals second-order kinetics at the high protein/phospholipid ratio, suggesting that this process is more efficient when the ICM particle density of ICM intramembranous complexes is high (107); (vi) the proton adenosine triphosphatase shows cyclical fluctuations in its activity despite the continued increase in adenosine triphosphatase antigen (58).

The enzymes involved in phospholipid synthesis are localized exclusively in the CM in both chemoheterotrophically and photoheterotrophically growing cells (15). Thus, the cell cycle-specific accumulation of phospholipid in the ICM described above must be regulated by controlling the movement of phospholipid into the ICM as opposed to regulating phospholipid biosynthetic enzyme activities localized within the ICM. Because phospholipid transfer activities have been discovered and purified from *R. sphaeroides* (26, 112), these might also be considered to play a role in phospholipid movement into the ICM. In this regard, recent studies have demonstrated that chromatophores with a high protein/phospholipid ratio (i.e., those isolated from cells just prior to cell division) are better substrates in the in vitro phospholipid transfer activity assay than those purified from cells just after cell division, which have a lower protein/phospholipid ratio (111).

From the studies of synchronous cell populations, it is clear that the number of photosynthetic units per chromatophore increases during the cell cycle, which suggests that within defined limits the density of ICM fixed photosynthetic units is related to the protein/phospholipid ratio. Examination of steady-state asynchronously grown cells at different light intensities shows that the apparent density of photosynthetic units per chromatophore is relatively constant (129). This is also apparent from the data in Table 1 which
demonstrate that the amounts of RC and B875 polypeptides do not vary when we compare equal amounts of chromatophores isolated from *R. sphaeroides* 2.4.1 grown at different light intensities. Decreases in the incident light intensity of growth result in increases in the cellular content of the ICM as does de novo ICM synthesis, which occurs when highly aerated cells are shifted to photosynthetic growth under nongratuitous conditions (see below). In light of these observations, there must be some regulatory mechanism that couples the number or formation of “new” ICM invaginations to synthesis of fixed photosynthetic units which is not merely dependent on the insertion of these complexes into the membrane. The recent demonstration of a mutant of *R. sphaeroides* which contains invaginations only at the cell poles and in which new invaginations only appear to arise at the site of cell constriction during division may prove useful in addressing questions pertaining to the process of ICM formation (67a). However, freeze-fracture analysis of wild-type cells undergoing a transition from aerobic to photosynthetic conditions did not show a localization of ICM formation at the cell poles (16).

**Induction and Assembly of the ICM**

The use of environmental shifts is a common experimental approach that has been used to study assembly of the ICM. This approach has been useful in defining, at the cellular level, a hierarchy of controls directed by various environmental factors. For example, lowering the $pO_2$ of a chemoheterotrophically growing culture (representing a gratuitous shift) is sufficient to trigger the induction of ICM development (63). Under these conditions, accumulation of B875 and RC complexes (69), as well as the mRNA for these pigment-protein complexes, is detected prior to synthesis of the B800-850 complexes (60). In contrast, both mRNA (Lee and Kaplan, unpublished results) and all three spectral complexes appear coordinately in the ICM when chemoheterotrophically grown cells are shifted to stringent nongratuitous anaerobic photosynthetic conditions (16). This suggests that synthesis of the B800-850 complexes responds differentially with respect to cultural oxygen tensions when compared with B875 and RC complexes. Therefore, it is of interest to understand how synthesis and assembly of the ICM and its associated functional complexes are integrated and controlled at both the molecular and genetic levels under these two distinct conditions of ICM induction. However, it has only recently been possible to address the control of ICM formation at this level due to the identification of the structural genes for several of the ICM components.

The occurrence and significance of phospholipid movement into the newly synthesized ICM were demonstrated during the process of de novo ICM induction under nongratuitous conditions (16). In the absence of net phospholipid synthesis or increase in cell number, new invaginations of the CM were seen early during the nearly 10- to 12-h lag prior to the resumption of growth under photosynthetic conditions (16). Moreover, the first invaginations observed during this lag period were much larger, irregular in shape, and more lipid enriched than mature ICM. The newly formed ICM invaginations may be related to a membrane fraction, which has been proposed to represent initiation sites for new ICM invaginations (60). This membrane fraction isolated by Dienemann and co-workers from cells undergoing a gratuitous shift has been designated as the upper pigmented band (UPB) due to its behavior on sucrose gradients (60), although it is not clear what percentage of the total cell membrane this UPB represents. The UPB fraction appears to be preferentially enriched in B875 and RC complexes, and it has been suggested that B800-850 complexes are also coordinately inserted with B875 and RC complexes into this membrane domain (60). The kinetics of insertion of pigment-protein complexes into the UPB parallel that observed during de novo synthesis of new ICM invaginations under gratuitous conditions (60). Perhaps related to this is the fact that an *R. sphaeroides* mutant deficient in B800-850 complexes and carotenoids (RS104) does not make mature ICM, but rather synthesizes long tubular ICM (67a). It is interesting to speculate that the tubular ICM formed in this mutant represents unrestricted nascent invaginations derived from the initial large lipid-enriched invaginations described by Chory et al. (16) or that they may be related to the UPB fraction (60). However, the tubular ICM in RS104 differs from the nascent invaginations in wild-type cells in that they extend from the cell poles and are unable to “mature.” Presumably due to the absence of B800-850 complexes.

Induction of the ICM, in addition to requiring the precise regulation of specific transcriptional activities, necessitates the coordinate assembly of individual spectral complexes prior to or concomitant with their incorporation into the ICM. Although these events are ultimately linked by as yet undefined mechanisms in wild-type bacteria, analysis of mutants defective in various aspects of ICM synthesis reveals that uncoupling of these processes is possible. For example, mutants containing a kanamycin resistance cartridge in either the *puh* (L. Sockett and S. Kaplan, unpublished results) or *paf* (37) operon of *R. sphaeroides* are unable to grow photosynthetically, but are able to synthesize ICM when grown in gratuitous conditions of anaerobic dark respiration. Thin-section electron microscopy of these mutants showed that the Pu* mutant contained somewhat irregular but vesicular shaped ICM, whereas the Pu- mutant appeared more like the wild type grown under similar conditions (Varga and Kaplan, unpublished results). In contrast, *N*-methyl-*N*-nitro-*N*-nitrosoguanidine-induced mutants, which are only lacking one of the LH complexes, are able to grow photosynthetically, and examination of the ICM from a B875 strain shows morphologically wild-type ICM invaginations (67a). As mentioned above, mutants lacking B800-850 complexes are still able to form invaginations, but these are somewhat distorted and are tubular in appearance. Thus, the analysis of both wild-type and mutant strains independently confirms that the signal(s) for phospholipid movement required for ICM formation is independent of the synthesis of spectral complexes and that the process of ICM invagination or maturation is separable from the insertion of individual intact spectral complexes. These observations also suggest that mature vesicular invaginations, characteristic of steady-state photoheterotrophic cells, are highly dependent upon the presence of B800-850 complexes and perhaps carotenoids.

However, once the process of ICM invagination begins in wild-type cells, assembly of mature ICM appears to be coupled to insertion of Bchl-protein complexes. That the H polypeptide of the RC is present in the CM of chemoheterotrophically grown cells (16) and that its total cellular abundance in photoheterotrophically grown cells is in excess of RC-M and RC-L (Table 1) lead to the suggestion that RC-H may serve a critical role in permitting RC-M and RC-L to enter the membrane at a particular point (docking) or to orient themselves (scaffolding) one to the other around the one membrane-spanning helix of the RC-H subunit. Subsequent events, such as the positioning of the B875 complex
relative to the RC, could depend upon the presence of either an intact RC complex or components of the RC as well as other posttranscriptional or posttranslational factors. This is supported by the observation that insertion of a kanamycin cartridge into a *pufA* deletion results in the reduction of RC (1%) as well as the loss of B875 complexes but not B800-850 complexes (Sackett and Kaplan, unpublished results). Cross-linking studies of *R. capsulatus* ICM has independently revealed a relationship between RC-H and B875 subunits (91). Finally, the assembly of the B800-850 complexes around the fixed photosynthetic unit consisting of B875 and RC complexes can be considered to be independent of RC and B875 spectral complexes, since the amount of B800-850 complex in the variable photosynthetic unit is tightly regulated by light intensity in wild-type cells and can be uncoupled from RC and B875 synthesis in mutant strains. Moreover, the above-described studies with specific mutant strains would suggest that the presence of B800-850 complexes in the ICM may be linked to the formation of mature invaginations but not dependent on the presence of RC or B875 complexes. Table 1 and Fig. 8 demonstrate that changes in the amount of Bchl per milligram of cell observed in *R. sphaeroides* 2.4.1 grown photosynthetically under different light intensities are reflected in both the amount of ICM per cell and the size of the variable photosynthetic unit. The cellular level of B800-850 complex relative to the B875 complex is greater when comparing cells grown at 10 W/m² versus those grown at 100 W/m² than when comparing cells grown at 10 W/m² versus those grown at 3 W/m². Thus, we suggest that the greatest contribution to the increase in Bchl per milligram of protein when comparing cells grown at 10 W/m² and those grown at 3 W/m² is primarily the result of an increase in the amount of ICM per cell and not the result of changes in the size of the variable photosynthetic unit. In turn, the presence and abundance of all spectral complexes in wild-type or mutant strains depend on the steady regulated synthesis of Bchl.

In wild-type cells the observation that the number of RC complexes per unit area of ICM is relatively constant and independent of light intensity (129) suggests that, once the process of ICM invagination takes place, the intracellular level of such invaginations is tightly linked to the cellular level of RCs. However, as previously stated, the ability to form new invaginations during de novo ICM induction does not depend upon the presence of intact RC complexes or the RC-H subunit since this appears to occur normally in strains lacking RC-H antigen. Future studies will be required to address whether other specific membrane proteins or phospholipid domains within the membrane are involved in the formation of ICM invaginations.

One point worth considering is that secondary structure predictions (122) and X-ray crystallography data indicate that the bulk of the RC-H polypeptide protrudes into the cytoplasm of the cell. The orientation of the RC-H subunit in the bilayer may make it available for imparting directionality for RC-L and RC-M insertion, alignment, and assembly of a functional RC pigment-protein complex. This protrusion of the RC-H subunit could also provide a mechanism by which the RC complex can signal or communicate directly with the cellular interior. For example, if RC complexes in an oxidized versus ground state gave rise to different conformational states of the cytoplasmic domain of the RC-H subunit, such conformational changes could be involved in activities such as regulating gene expression or bacterial tactic responses by light and oxygen. Such a sensing mechanism would be analogous to the response of other bacterial membrane proteins involved in responding to changes in environmental stimuli (97, 126). For example, it is possible that the RC-H polypeptide may be functionally similar to the recently described class of transmembrane sensor proteins which provide bacteria a mechanism by which they can sense and respond to changes in their environment (i.e., osmolarity changes, phosphate or nitrogen limitation) (97, 126). By analogy with these other two component systems, there may also be a positive regulatory protein which interacts directly with the transmembrane protein to transmit some oxygen or light-regulated signal, resulting in either direct or indirect transcriptional activation of the genes under its control.

**REGULATION OF PHOTOSYNTHETIC GENE EXPRESSION**

In an effort to keep this review as current as possible, this section contains some unpublished results. We trust the reader will distinguish our unpublished observations as preliminary.

The structural genes for the RC and LH polypeptides have all been placed within transcriptional units in *R. sphaeroides* (40, 67, 139, 140) and *R. capsulatus* (7, 137, 138), and recent experiments in both bacteria have demonstrated that ICM induction under anaerobic conditions is not solely regulated at the transcriptional level (see below).

**Expression of the puf Operon**

The *puf* operon (Fig. 7) represents the most thoroughly studied transcriptional unit encoding Bchl-binding proteins, and in *R. sphaeroides* it encodes two stable polycistronic mRNA molecules: a high-abundance small transcript (approximately 600 nucleotides [nt]), specific for the *pufB* and *pufA* genes, which has a 5'-end 104 nt upstream of *pufB*; and a low-abundance large transcript (2,600 nt) which encodes *pufB* and *pufM* and which has a 5'-end 75 nt upstream of *pufB*
More recent results demonstrate that the large puf transcript extends far enough to encode a putative downstream gene, pufX (Gyure and Donohue, personal communication), which was first described in *R. capsulatus* (132). The ratio of small/large puf operon-specific transcript varies from 20 to 30:1 in chemoheterotrophically grown *R. sphaeroides* to 8 to 15:1 in photosynthetically grown cells (139, 140). Therefore, the obvious question arises as to the origin of each of these puf operon-specific transcripts. Belasco et al. (7) working with *R. capsulatus* have suggested specific 3'
processing of the 2.600-nt-long \textit{pufBAMX} transcript to yield a steady-state level of 9:1 \textit{pufBA}/\textit{pufBAMX} mRNA species. In \textit{R. sphaeroides} it has been proposed that a stem-loop which could be formed in the intercistronic region (124 base pairs) between \textit{pufBA} and \textit{pufLMX} serves as a differential transcription terminator so that the ratio of these two transcripts result from the selective termination of mRNA species with unique 5' ends upstream of \textit{pufB} (66). Deletion of the purported terminator region in \textit{R. sphaeroides} yielded a mutant that displays a B875 \textsuperscript{−} phenotype which grows photosynthetically (Dehoff and Kaplan, unpublished results). This phenotype is consistent with the intercistronic region functioning as a transcription terminator, although more work is required to establish the validity of this hypothesis.

Observations from our laboratory have identified a DNA sequence containing a 20-amino-acid open reading frame (orfK) immediately upstream of the \textit{pufB} structural gene. This putative ‘leader’ polypeptide coding region is preceded by a Shine-Dalgarno sequence, has an ATG-methionine, and terminates with a UAG one base prior to the ATG of the \textit{pufB} gene. Characteristic of the DNA sequence in this region is the use of relatively rare codons for 9 of the 20 designated amino acids as well as the use of a UAG codon as a terminator, which has not been previously observed in any of the \textit{R. sphaeroides} structural genes sequenced so far. Previous studies have documented differences in transfer RNA isoaccepting species in chemoheterotrophically and phototrophically grown cells (101), and experiments are under way to test whether this proposed leader peptide functions to control translation of \textit{pufB}-mRNA in chemoheterotrophic cells, perhaps by ‘ribosome stalling’ resulting in the inability to translate \textit{pufB}-specific mRNA under aerobic conditions but not under photosynthetic conditions. It is also possible that the ratio of small/large \textit{puf} operon transcripts may be influenced by ribosome stalling and could be coupled to differential transcription termination in the intercistronic region between \textit{pufA} and \textit{pufL}. It is evident that the \textit{puf} operon genetic region may be highly regulated by several levels of control in \textit{R. sphaeroides}.

Since the \textit{puf} operon codes for essential gene products required in precise stoichiometries for the light reactions of photosynthesis, it is perhaps not surprising that expression of this operon is highly regulated by physiological conditions. In addition to allowing us to study the mechanisms of gene expression in these bacteria, the analysis of how expression of this operon is regulated has provided the opportunity to study how the stoichiometry, assembly, and organization of a supramolecular membrane complex are regulated and, thus, coupled to other aspects of cellular metabolism required for photosynthesis. The ratio of the small/large \textit{puf} operon-specific transcripts (approximately 8 to 15:1) is essentially constant under all photosynthetic growth conditions (Kaplan, unpublished results), and it has been proposed that this excess of small to large \textit{puf} transcript results in the 10- to 15-fold excess of B875 to RC complexes in the ICM (1. 139, 140). Although in both \textit{R. sphaeroides} and \textit{R. capsulatus} the 5' ends of the \textit{puf} transcripts have been mapped to regions immediately upstream of \textit{pufB} (7, 140), recent genetic evidence suggests that a region several hundred base pairs upstream of \textit{pufB} in \textit{R. capsulatus} is required in \textit{cis} to obtain regulated expression of this operon in vivo (6). This region codes for a 77-amino-acid polypeptide, designated \textit{Q} gene product (Bauer and Marrs, Abstr. Mol. Biol. Photosynth. Procaryotes. 1987, p. 2). Therefore, in \textit{R. capsulatus} it has been proposed that the \textit{Q} gene is transcriptionally linked to the \textit{puf} operon and transcripts which initiate upstream of \textit{Q} are processed rapidly in vivo to produce the observed 5' ends associated with each stable \textit{puf} operon transcript. Moreover, the absence of detectable transcript derived from \textit{Q} under chemoheterotrophic growth conditions but the presence of substantial amounts of both \textit{puf}-specific transcripts under these same conditions would imply an additional requirement for the differential regulation of the mRNA processing function under aerobic versus photosynthetic growth (Lee and Kaplan, unpublished results). To date, there is no physical evidence linking the \textit{Q} gene transcript to either of the \textit{puf} operon-specific transcripts.

Recent genetic evidence in \textit{R. capsulatus} demonstrates that insertions into the \textit{Q} gene result in a dramatic decrease of Bchl or any precursors in the Mg branch of this pathway (Bauer and Marrs, Abstr. Mol. Biol. Photosynth. Procaryotes, 1987, p. 2). These and other results have led Bauer and Marrs to suggest that this gene product functions as a carrier for Bchl intermediates, an activity previously suggested by Lascelles to exist in \textit{R. sphaeroides} (71, 96). In \textit{R. sphaeroides}, deletion of the \textit{Q} gene resulting in retention of approximately 25% of the proximal region of \textit{Q} resulted in Bchl and carotenoid synthesis at greatly reduced levels compared with that of wild type (37). A second open reading frame designated \textit{R} in \textit{R. sphaeroides} maps upstream of the \textit{Q} gene (Havelka and Kaplan, unpublished results), and complementation analysis of a mutant deficient in B875 complexes (RS103: 61. 76) showed that this mutant could be restored to wild type with the \textit{R} gene region in trans (J. Davis and S. Kaplan, unpublished results). Furthermore, complementation experiments have shown that this same region can restore the previously described \textit{pshA} deletion/insertion mutant which is phenotypically B875 \textsuperscript{−} RC \textsuperscript{−} to B875 \textsuperscript{−} when grown anaerobically in the dark on dimethyl sulfoxide, independent of restoring functional RC complexes (Sokett and Kaplan, unpublished results). Since we know that the RS103 mutant is not affected in \textit{puf} operon transcription (Davis and Kaplan, unpublished results), these data imply that the \textit{R} gene product (or a closely linked region) functions in an as yet uncharacterized role to control synthesis or assembly specifically of the B875 complexes and also suggest that expression of the \textit{puf} operon or DNA sequences linked to \textit{puf} can influence assembly of \textit{puf} operon gene products, perhaps by regulating expression of the \textit{R} gene product.

Further complicating the analysis of the expression of the region upstream of \textit{pufB} is determination of the location of the promoter for the \textit{puf} operon. If it is just upstream of the 5' ends of the stable \textit{puf} operon transcripts (7, 140), then the promoter would be located in the distal portion of the reading frame of \textit{Q}. Similarly, the promoter for the \textit{Q} gene would be within the distal portion of the \textit{R} coding region. Therefore, if there are \textit{cis}-acting effects due to the physical linkage of these genes, it is difficult to distinguish between these effects and transcriptional linkage of these genes with the \textit{puf} operon. Recent results in \textit{R. sphaeroides} reveal that lacZ fusions into \textit{pufB} and extending from the distal portion of \textit{Q} can express \textbeta-galactosidase activity in vivo (Havelka and Kaplan, unpublished results). Similarly, ‘runoff’ transcription assays utilizing highly purified RNA polymerase from \textit{R. sphaeroides} map transcription start sites for \textit{Q} to the distal region of \textit{R} and for \textit{puf} just downstream of \textit{Q} (Kansy and Kaplan, in preparation). In vitro transcription-translation experiments confirmed these results for sequences required for expression of the \textit{puf} operon (66).
Expression of the puc Operon

Of all of the genes specifying Bchl-binding proteins which have been analyzed to date (40, 67, 137–139), transcription of the puc operon appears to be regulated to the greatest extent by changes in physiological conditions. Furthermore, mutations which affect the synthesis of the RC and B875 polypeptides produce increased levels of puc mRNA under anaerobic respiratory conditions with dimethyl sulfoxide, although this is not necessarily accompanied by corresponding increases in the levels of B800-850 complexes (37). It is not known whether derepression of puc mRNA levels in these mutants is accompanied by increased synthesis of cellular levels of unliganded B800-850 polypeptides. That one sees derepression of puc mRNA levels in such mutant strains grown under anaerobic respiration in the dark suggests that factors other than light intensity can affect puc operon expression at the transcriptional or posttranscriptional level (i.e., mRNA stability). Therefore, regulation of puc operon expression by light intensity in the wild-type strain could be an indirect effect and may be related more directly to one of the following: (i) movement of electrons through the electron transport chain; (ii) the redox state of the cell; (iii) the levels of puf or puh gene products in the cell; (iv) the available pool of Bchl; (v) some as yet unknown regulatory precursor.

Posttranscriptional Control

As might have been expected from the analysis of mutants described above, there are transcriptional and translational differences related to the expression of the structural genes encoding the polypeptides of the different spectral complexes. The steady-state levels of the mRNA encoding many of the photosynthetic genes have been reported from a variety of physiological conditions (40, 67, 137–139). For example, the two stable (1,400 and 1,130 nt) puhA (40), pufBA and pufBALMX (139) transcripts are readily detectable in R. sphaeroides grown chemoheterotrophically in a 30% O₂ atmosphere, whereas puc (640 nt) mRNA is barely apparent (67). As mentioned earlier, the puhA gene product (RC-H polypeptide) is present in the CM when grown under high O₂ conditions, although there are no detectable spectral complexes (16, 40). Moreover, no RC-M, RC-L (16), B875-α (67a), or B800-850-β (16) polypeptides could be detected in a Western blot (immunoblot) analysis of either soluble or membrane fractions derived from R. sphaeroides grown in a 30% O₂ atmosphere. This implicates physiologically regulated translational or posttranslational control over the synthesis of individual ICM proteins in addition to different transcriptional effects being involved in regulating the steady-state level of these mRNAs under different physiological conditions. One possible explanation for the lack of the pigment-binding polypeptides under chemoheterotrophic growth conditions is due to instability of the Bchl-binding gene products in the absence of their ligand. Bchl. Support for this model comes from recent pulse-labeling experiments in R. capsulatus which have demonstrated the existence of a transient pool of pigment-binding polypeptides under conditions of induction for ICM synthesis in either Bchl mutants or in wild-type cells in the presence of inhibitors of porphyrin biosynthesis (39, 70). In R. sphaeroides, the lability of both the B800-850-β (67) and B875-β (Kiley, Ph.D. thesis) polypeptides synthesized in the absence of Bchl in a coupled in vitro transcription-translation system has been demonstrated. Both observations are consistent with a model in which synthesis of these polypeptides is also controlled posttranslationally, perhaps as the result of the action of a specific protease which acts on the unliganded pigment-binding polypeptides. However, it still remains to be determined whether puf-specific mRNA is translated in vivo under chemoheterotrophic conditions. Although these data suggest a posttranslational regulation for the mechanism of these gene products in the absence of Bchl, additional forms of translational control (e.g., Bchl-dependent translation, ribosome stalling) over photosynthetic gene expression cannot be ruled out.

There is at least three- to sixfold more puf- and puh-specific mRNA in photosynthetically grown (100 W/m²) R. sphaeroides than in chemoheterotrophically grown cells (40, 139). In contrast, the amount of puc-specific mRNA is at least 100-fold greater in photosynthetically grown cells than measured in chemoheterotrophically grown cells in the presence of 30% O₂ (Lee and Kaplan, unpublished results). The amount of each of these transcripts, in steady-state photosynthetically grown cells, varies two- to fivefold depending on the incident light intensity of growth. In general, changes in puc, puf, and puh operon-specific mRNAs in wild-type cells grown under steady-state photosynthetic conditions closely paralleled the amount of each cognate spectral complex present within the ICM (Table 1). However, more work is required before we understand the precise physiological relationship between mRNA levels and the cellular abundance of specific spectral complexes in both wild-type and mutant strains. It is not known whether the regulation of an individual spectral complex (RC, B875, or B800-850) under photosynthetic conditions at different light intensities is due solely to changes in transcription of these operons or whether the mRNAs observed in wild-type or mutant strains (see above) are due to a combination of transcription initiation and differential message stability.

CONCLUSIONS

The recent identification and sequencing of the structural genes for the Bchl-binding proteins and components of the electron transport chain in the photosynthetic bacteria have been preceded by a strong foundation in the biochemical, spectroscopic, and genetic analyses of these organisms. These recent accomplishments provide the framework to probe structure-function relationships of the above-described membrane complexes, especially when three-dimensional crystal structures are available. Use of molecular genetic techniques in our analysis of the photosynthetic bacteria also has the potential to unravel the complex molecular mechanisms by which these organisms sense and respond to environmental stimuli such as oxygen and light. This review highlights the need to expand the pioneering work of Lascelles and co-workers on the pathway for tetrapyrrole biosynthesis in these bacteria. Without such analyses, it will be difficult, if not impossible, to completely understand how the synthesis of ligands such as heme, Bchl, carotenoids, and bacteriopheophytin is coupled to synthesis or assembly or both of ICM apoproteins into functional units.

Further, assembly and function of individual ICM components cannot be separated from the synthesis of the membrane bilayer in which they reside. Many unanswered questions still persist with regard to the processes or events involved in formation and partitioning of ICM invaginations during cell division in both steady-state cells or cells responding to changes in environmental conditions. Although
there is recent genetic evidence suggesting that specific proteins function in assembly of Bchl-protein complexes, much work is needed to precisely define their physiological role(s), subcellular location, and mechanism of action. The recent crystalization of RC complexes in *Rhodopseudomonas viridis* and *R. sphaeroides* offers the potential for crystallization of other membrane protein complexes which will allow us to understand how individual redox components function and interact with other such complexes in the membrane. It is perhaps not surprising that specific structural or regulatory gene mutations have pleiotropic effects on the structure, assembly, and regulation of the ICM since all of these components reside in the same lipid bilayer.

Still one of the most intriguing questions is how all of these particular components are targeted to the ICM to create this specific membranous domain. The fact that photoheterotrophically growing cells contain three distinct membrane systems (ICM, cell membrane, and outer membrane), each of which possesses its own unique macromolecular composition and structure, raises important questions. How do proteins unique to each membrane system find their correct location and how do proteins which are present in more than one membrane system partition themselves between these membranes? The mode of photosynthetic membrane assembly also poses many questions with regards to the physical state of the bilayer and the function of specific proteins, lipids, or membrane domains in controlling activity or diffusion of components between the cell membrane and the ICM.

Studies of the structure, function, and expression of individual components within the inducible photosynthetic apparatus of *R. sphaeroides* and in the photosynthetic bacteria in general have reached a high degree of sophistication. Although not discussed here, events occurring at the level of the ICM must somehow be communicated to regulate the expression of those soluble activities which we associate with photosynthetic carbon assimilation (Calvin cycle enzymes) and with nitrogen fixation. Given the rate of progress over the past several years, the next few years will prove to be exciting, informative, and, doubtless, somewhat controversial. However, it is our hope that we do not lose site of the remarkable versatility and metabolic elegance of the photosynthetic bacteria as models with which to address significant problems in many areas of biological interest.

**ACKNOWLEDGMENTS**

We acknowledge the support provided by research grants from the Public Health Service National Institute of General Medical Sciences, National Science Foundation, U.S. Department of Agriculture, and Amoco Corp.

We also acknowledge those individuals willing to share their results with us and to include their data in this review. Finally, we thank those members of this laboratory, past and present, who have made these studies a great deal more than just work.

**ADDENDUM IN PROOF**

Recent analysis of an *R. sphaeroides* mutant containing a deletion of the intercistronic region between *pufA* and *pufL* shows reduced amounts of B875 (7%) and RC (25%) spectral complexes (R. Prince, personal communication) and similar decreases in the B875-a (5%) and RC (60%) polypeptides by Western blot analysis (B. Dehoff and S. Kaplan, unpublished results). The physiological consequence of this deletion is that the ratio of B875 to RC complexes has been decreased to approximately 1:2:1. This appears to result from altered stoichiometry of the small to large *puf* operon mRNA, perhaps due to a defect in transcription termination between *pufA* and *pufL* (see text). Recently, Klug et al. have also shown that deletion of the analogous intercistronic region in *R. capsulatus* also affects the stoichiometry of the *puf* operon gene products, and they conclude that this supports 3’ processing of the large *puf* operon mRNA as the origin of the small transcript rather than transcription termination (67b). Such strains will provide the basis for studying the mechanism underlying the stoichiometric synthesis of *puf* operon gene products (mRNA and polypeptides), as well as the effect that altered ratios of these Bchl-protein complexes have on the light reactions of photosynthesis.

The region upstream of *pufB* has been altered by site-directed mutagenesis such that the putative *orfK* gene product was fused in frame to the *pufB* protein. The predicted fused gene product can be detected in the *R. sphaeroides* in vitro transcription-translation system, suggesting that *orfK* has the proper signals required for translation. The wild-type *pufB* gene product was also synthesized in great molar excess over the fused gene product in vitro, indicating that there could be differences in translation initiation at the *orfK* and *pufB* ribosome-binding site. Thus, it is possible that translation of the *orfK* region can control transcription termination between *pufA* and *pufL* (see text). Analysis of the physiological effects of mutations in *orfK* in vivo will aid in addressing the role of the *orfK* gene on *puf* operon expression and synthesis of the fixed photosynthetic unit.

**LITERATURE CITED**

1. Aagaard, J., and W. R. Sistrom. 1972. Control of synthesis of reaction center bacteriochlorophyll in photosynthetic bacteria. Photochem. Photobiol. 15:209–225.

2. Agalidis, I., A. M. Nuijts, and F. Reiss-Husson. 1987. Characterization of an LM unit purified by affinity chromatography from *Rhodobacter sphaeroides* reaction centers and interaction with the H subunit. Biochim. Biophys. Acta 900:242–250.

3. Allen, J. P., G. Feher, T. O. Yeates, H. Komiyama, and D. C. Rees. 1987. Structure of the reaction center from *Rhodobacter sphaeroides* R-26: the protein subunits. Proc. Natl. Acad. Sci. USA 84:6162–6166.

4. Allen, J. P., G. Feher, T. O. Yeates, D. C. Rees, J. Deisenhofer, H. Michel, and R. Huber. 1986. Structural homology of reaction centers from *Rhodopseudomonas sphaeroides* and *Rhodopseudomonas viridis* as determined by X-ray diffraction. Proc. Natl. Acad. Sci. USA 83:8589–8593.

5. Ashby, M. K., S. A. Coomber, and C. N. Hunter. 1987. Cloning, nucleotide sequence, and transfer of genes for the B800–850 light-harvesting complex of *Rhodobacter sphaeroides*. FEBS Lett. 213:245–248.

6. Bauer, C. E., M. Eleuterio, D. A. Young, and B. L. Marrs. 1987. Analysis of transcription through the *Rhodobacter capsulatus* puf operon using a translational fusion of *pufM* to the *E. coli lacZ* gene. p. 699–705. In J. Biggins (ed.). Progress in photosynthesis research. vol. 4. Martinus Nijhoff Publishers, Dordrecht. The Netherlands.

7. Belasco, J. G., J. T. Beatty, C. W. Adams, A. V. Gabain, and S. N. Cohen. 1985. Differential expression of photosynthesis genes in *R. capsulata* results from segmental differences in stability within the polycistronic *rvaA* transcript. Cell 40:171–181.

8. Berard, J., G. Belanger, P. Corriveau, and G. Gingras. 1986. Molecular cloning and sequence of the B880 holochrome genes from *Rhodospirillum rubrum*. J. Biol. Chem. 261:82–87.

9. Biel, A. J., and B. L. Marrs. 1983. Transcriptional regulation of several genes for bacteriochlorophyll biosynthesis in *Rhodo- pseudomonas capsulata* in response to oxygen. J. Bacteriol. 156:686–694.

10. Broglio, R. M., C. N. Hunter, P. Delepelaire, R. A. Niederman,
N.-H. Chua, and R. K. Clayton. 1980. Isolation and characterization of the pigment-protein complexes of Rhodopseudomonas sphaeroides by lithium dodecylsulfate/polyacrylamide gel electrophoresis. Proc. Natl. Acad. Sci. USA 77:87–91.
11. Brown, A. E., F. A. Eiserling, and J. Lascelles. 1972. Bacteriochlorophyll synthesis and the ultrastructure of wild type and mutant strains of Rhodopseudomonas sphaeroides. Plant Physiol. 50:743–746.
12. Brown, A. E., and J. Lascelles. 1972. Phytol and bacteriochlorophyll synthesis in Rhodopseudomonas sphaeroides. Plant Physiol. 50:747–749.
13. Brumisholz, R. A., H. Zuber, J. Valentine, J. G. Lindsay, K. J. Wooley, and R. J. Coggell. 1986. The membrane location of the B890-complex from Rhodospirillum rubrum and the effect of carotenoid on the conformation of its two apoproteins exposed at the cytoplasmic surface. Biochim. Biophys. Acta 849:295–303.
14. Cain, B. D., C. Deal, R. T. Fraley, and S. Kaplan. 1981. In vivo intermembrane transfer of phospholipids in the photosynthetic bacterium Rhodopseudomonas sphaeroides. J. Bacteriol. 145:1154–1166.
15. Cain, B. D., T. J. Donohue, W. D. Shepherd, and S. Kaplan. 1984. Localization of phospholipid biosynthetic enzyme activities in cell-free fractions derived from Rhodopseudomonas sphaeroides. J. Biol. Chem. 259:942–948.
16. Chory, J., T. J. Donohue, A. R. Varga, L. A. Staelin, and S. Kaplan. 1984. Induction of the photosynthetic membranes of Rhodopseudomonas sphaeroides: biochemical and morphological studies. J. Bacteriol. 159:540–554.
17. Chory, J., and S. Kaplan. 1982. The in vitro transcription-translation of DNA and RNA templates by extracts of Rhodopseudomonas sphaeroides. J. Biol. Chem. 257:15110–15121.
18. Chory, J., and S. Kaplan. 1983. Light-dependent regulation of the synthesis of soluble and intracytoplasmic membrane proteins of Rhodopseudomonas sphaeroides. J. Bacteriol. 153:465–474.
19. Chory, J., E. D. Muller, and S. Kaplan. 1985. DNA-directed in vitro synthesis and assembly of the II d-ribulose-1,5-bisphosphate carboxylase/oxygenase from Rhodopseudomonas sphaeroides. J. Bacteriol. 161:307–313.
20. Clark, W. G., E. Davidson, and B. L. Marrs. 1984. Variation of levels of mRNA coding for antenna and reaction center polypeptides in Rhodopseudomonas capsulata in response to changes in oxygen concentration. J. Bacteriol. 157:945–948.
21. Clayton, R. K., and B. J. Clayton. 1978. Properties of photochemical reaction centers purified from Rhodopseudomonas gelatinosa. Biochim. Biophys. Acta 501:470–477.
22. Clement-Metral, J. D. 1979. Activation of ALA synthase by reduced thioredoxin in Rhodopseudomonas sphaeroides Y. FEBS Lett. 101:116–120.
23. Clement-Metral, J. D., J.-O. Hoog, and A. Holmgren. 1986. Characterization of the thioredoxin system in the facultative phototroph Rhodobacter sphaeroides Y. Eur. J. Biochem. 161:119–126.
24. Coggell, R. J., and A. R. Crofts. 1978. Analysis of the pigment protein content of an antenna pigment-protein complex from three strains of Rhodopseudomonas sphaeroides. Biochim. Biophys. Acta 502:409–416.
25. Coggell, R. J., and J. P. Thornber. 1980. Light-harvesting pigment-protein complexes of purple photosynthetic bacteria. FEBS Lett. 122:1–8.
26. Cohen, L., D. Lueking, and S. Kaplan. 1979. Intermembrane phospholipid transfer mediated by cell-free extracts of Rhodopseudomonas sphaeroides. J. Biol. Chem. 254:721–728.
27. Cohen, L. K., and S. Kaplan. 1981. The non-detergent solubilization and isolation of intracytoplasmic membrane polypeptides from Rhodopseudomonas sphaeroides. J. Biol. Chem. 256:5901–5908.
28. Cohen, L. K., and S. Kaplan. 1981. Characterization of the three major intracytoplasmic membrane polypeptides isolated from Rhodopseudomonas sphaeroides. J. Biol. Chem. 256:5909–5915.
29. Cohen-Bazire, G., W. R. Sistrom, and R. Y. Stanier. 1957. Kinetic studies of pigment synthesis by non-sulfur purple bacteria. J. Cell. Comp. Physiol. 49:25–68.
30. Comber, S. A., M. F. Ashby, and C. N. Hunter. 1987. Cloning and oxygen regulated expression of genes for the bacteriochlorophyll biosynthetic pathway in Rhodopseudomonas sphaeroides. p. 737–740. In J. Biggins (ed.). Progress in photosynthesis research, vol. 4. Martinus Nijhoff Publishers, Dordrecht, The Netherlands.
31. Crofts, A. R., S. W. Meinhardt, and J. R. Bowyer. 1982. The electron transport chain of Rhodopseudomonas sphaeroides, p. 477–498. In B. L. Trumpproer (ed.). Function of quinones in energy conserving systems. Academic Press, Inc., New York.
32. Crofts, A. R., and C. A. Wright. 1983. The electrochemical domain of photosynthesis. Biochim. Biophys. Acta 726:149–185.
33. Daldal, F. S., Cheng, J. Applebaum, E. Davidson, and R. Prince. 1986. Cytochrome c is not essential for photosynthetic growth of Rhodopseudomonas capsulata. Proc. Natl. Acad. Sci. USA 83:2012–2016.
34. Davidson, E. F., and R. J. Coggell. 1981. The polypeptide composition of the B850 light-harvesting pigment-protein complex from Rhodopseudomonas capsulata. FEBS Lett. 132:81–84.
35. Davidson, E. F., and F. Daldal. 1987. Primary structure of the bc1 complex of Rhodopseudomonas capsulata: Nucleotide sequence of the pet operon encoding the Rieske cytochrome b1 and cytochrome b5, apoproteins. J. Mol. Biol. 195:13–24.
36. Davidson, E. F., and F. Daldal. 1987. The pet operon, encoding the Rieske Fe-S protein, cytochrome b, and cytochrome c1 apoproteins previously described from Rhodopseudomonas sphaeroides is from Rhodopseudomonas capsulata. J. Mol. Biol. 195:23–29.
37. Davis, J. T., T. J. Donohue, and S. Kaplan. 1988. Construction, characterization, and complementation of a Pf mutant of Rhodobacter sphaeroides. J. Bacteriol. 170:320–329.
38. Deisenhofer, J., O. Epp, K. Miki, R. Huber, and H. Michel. 1985. Structure of the protein subunits in the photosynthetic reaction centre of Rhodopseudomonas viridis at 3Å resolution. Nature (London) 318:619–624.
39. Dierstein, R. 1984. Synthesis of pigment-binding protein in tolune-treated Rhodopseudomonas capsulata and in cell-free systems. Eur. J. Biochem. 138:509–518.
40. Donohue, T. J., J. H. Hoger, and S. Kaplan. 1986. Cloning and expression of the Rhodobacter sphaeroides reaction center H gene. J. Bacteriol. 168:953–961.
41. Donohue, T. J., and S. Kaplan. 1986. Synthesis and assembly of bacterial photosynthetic membranes, p. 632–639. In L. A. Staelin and C. J. Arnzen (ed.). Photosynthesis III: photosynthetic membranes. Encyclopedia of plant physiology, new series, vol. 19. Springer-Verlag, New York.
42. Donohue, T. J., A. G. McEwan, and S. Kaplan. 1986. Cloning, DNA sequence, and expression of the Rhodobacter sphaeroides cytochrome c5 gene. J. Bacteriol. 168:962–972.
43. Drews, G. 1986. Adaptation of the bacterial photosynthetic apparatus to different light intensities. Trends. Biochem. Sci. 11:255–257.
44. Drews, G., J. Peters, and R. Dierstein. 1983. Molecular organization and biosynthesis of pigment-protein complexes of Rhodopseudomonas capsulata. Ann. Microbiol. (Paris) 134B:151–158.
45. Drews, G., and R. Feick. 1978. Isolation and characterization of light-harvesting bacteriochlorophyll protein complexes from Rhodopseudomonas capsulata. Biochim. Biophys. Acta 501:499–513.
46. Drews, G., K. T. Hatmark, H. deKlerk, and M. Kamen. 1970. Isolation and chemical properties of two c-type cytochromes of Rhodospirillum molischianum. Biochemistry 9:1984–1990.
47. Fanica-Gaigner, M., and J. Clement-Metral. 1973. Cellular compartmentation of two species of δ-aminolevulinic acid synthetase in a facultative phototrophic bacterium Rps. rubrum. Biochim. Biophys. Res. Commun. 55:610–615.
48. Feher, G., and M. Y. Okamura. 1975. Chemical composition and properties of reaction centers, p. 349–386. In R. K.
Clayton and W. R. Sistrom (ed.). The photosynthetic bacteria. Plenum Publishing Corp., New York.

49. Flattmark, T., K. Dus, H. deKlerk, and M. Kamen. 1970. Comparative study of physiochemical properties of two c-type cytochromes of Rhodospirillum molischianum. Biochemistry 9:1991–1995.

50. Forneri, C. S., and S. Kaplan. 1982. Genetic transformation of Rhodopseudomonas sphaeroides by plasmid DNA. J. Bacteriol. 152:89–97.

51. Forrest, M. E., and J. T. Beatty. 1987. Purification of RNA polymerase and its use for in vitro transcription. FEBS Lett. 212:28–34.

52. Fryk, P., and S. Kaplan. 1971. Isolation and fractionation of the photosynthetic membrane organelles from Rhodopseudomonas sphaeroides. J. Bacteriol. 108:465–473.

53. Fraley, R. T., D. R. Leuking, and S. Kaplan. 1977. Intracytoplasmic membrane synthesis in synchronously dividing cell populations of Rhodopseudomonas sphaeroides; polyopptide insertion into growing membrane. J. Biol. Chem. 252:453–464.

54. Fraley, R. T., G.-S. L. Yan, D. R. Leuking, and S. Kaplan. 1979. The physical state of the intracytoplasmic membrane of Rhodopseudomonas sphaeroides and its relationship to the cell division cycle. J. Biol. Chem. 254:1987–1991.

55. Gabellini, N., J. R. Bowyer, E. Hurt, B. A. Melandri, and G. Hauska. 1982. A cytochrome b6/c complex with ubiquinol-cytochrome c reductase activity from Rhodopseudomonas sphaeroides. Eur. J. Biochem. 126:105–111.

56. Gabellini, N., V. Harnisch, J. E. G. McCarthy, G. Hauska, and W. Sebald. 1985. Cloning and expression of the fbc operon encoding the FeS protein, cytochrome b and cytochrome c, from the Rhodopseudomonad sphaeroides b6/c complex. EMBO J. 4:549–553.

57. Hoger, J., J. C. Chory, and S. Kaplan. 1986. In vitro biosynthesis and membrane association of photosynthetic reaction center subunits from Rhodopseudomonas sphaeroides. J. Bacteriol. 165:942–950.

58. Hoger, J. H., S.-P. Tai, and S. Kaplan. 1987. Membrane adenosine triphosphatase in synchronous cultures of Rhodobacter sphaeroides. Biochim. Biophys. Acta 908:70–80.

59. Imhoff, J. F., H. G. Truper, and N. Pfennig. 1984. Rearrangement of the species and genera of the phototrophic "purple nonsulfur bacteria." Int. J. Syst. Bacteriol. 34:340–343.

60. Inamine, G. S., J. van Houten, and R. A. Niederman. 1984. Intracellular localization of photosynthetic membrane growth initiation sites in Rhodopseudomonas sphaeroides. J. Bacteriol. 158:474–480.

61. Jackson, W. J., P. J. Kiley, C. E. Haith, S. Kaplan, and R. C. Prince. On the role of the light-harvesting B880 in the correct insertion of the reaction center of Rhodobacter capsulatus and Rhodobacter sphaeroides. FEBS Lett. 215:171–174.

62. Jones, O. T. G. 1978. Biosynthesis of porphyrins, hemes, and chlorophylls. p. 751–777. In R. K. Clayton and W. R. Sistrom (ed.). The photosynthetic bacteria. Plenum Publishing Corp., New York.

63. Kaplan, S. 1981. Development of the membranes of photosynthetic bacteria. Photochem. Photobiol. 34:769–774.

64. Kaplan, S., and C. J. Arntzen. 1982. Photosynthetic membrane structure and function. p. 65–151. In Govindjee (ed.). Photosynthesis: energy conversion by plants and bacteria. vol. 1. Academic Press, Inc., New York.

65. Kaplan, S., B. D. Cain, T. J. Donohue, W. D. Shepherd, and G. S. L. Yan. 1983. Biosynthesis of the photosynthetic membranes of Rhodopseudomonas sphaeroides. J. Cell. Biochem. 22:15–29.

66. Kiley, P. J., T. J. Donohue, W. A. Havelka, and S. Kaplan. 1986. DNA sequence and in vitro expression of the B875 light-harvesting polypeptides of Rhodobacter sphaeroides. J. Bacteriol. 169:742–750.

67. Kiley, P. J., and S. Kaplan. 1987. Cloning, DNA sequence, and expression of the Rhodobacter sphaeroides light-harvesting B850-850 and B850-850-550 genes. J. Bacteriol. 169:268–3375.

68. Kiley, P. J., A. Varga, and S. Kaplan. 1988. Physiological and structural analysis of light-harvesting mutants of Rhodobacter sphaeroides. J. Bacteriol. 170:1103–1115.

69. Klug, G., C. W. Adams, J. Belasco, B. Doerge, and S. N. Cohen. 1987. Biological consequences of segmental alterations in mRNA stability: effects of deletion of the intercistronic hairpin loop region of the Rhodobacter capsulatus puf operon. EMBO J. 6:3515–3520.

70. Klug, G., and G. Drews. 1984. Construction of a gene bank of Rhodopseudomonas capsulatus using a broad host DNA cloning system. Arch. Microbiol. 139:319–325.

71. Klug, G., N. Kaufmann, and G. Drews. 1985. Gene expression of pigment binding proteins of the bacterial photosynthetic apparatus: transcription and assembly in the membrane of Rhodopseudomonas capsulatus. Proc. Natl. Acad. Sci. USA 82:6485–6489.

72. Klug, G., R. Liebetanz, and G. Drews. 1986. The influence of bacteriochlorophyll biosynthesis on formation of pigment-binding proteins and assembly of pigment complexes in Rhodopseudomonas capsulatus. Arch. Microbiol. 146:284–291.

73. Lascelles, J. 1978. Regulation of pyrrole synthesis, p. 795–808. In R. K. Clayton and W. R. Sistrom (ed.). The photosynthetic bacteria. Plenum Publishing Corp., New York.

74. Leong, S. A., G. S. Ditt, and D. R. Helinski. 1982. Heme biosynthesis in Rhizobium. Identification of a cloned gene coding for 5-aminolevulinic acid synthetase from Rhizobium meliloti. J. Biol. Chem. 257:8724–8730.

75. Luchting, D. R., R. T. Fraley, and S. Kaplan. 1978. Intracytoplasmic membrane synthesis in synchronous cell populations of Rhodopseudomonas sphaeroides. J. Biol. Chem. 253:451–457.

76. Marrs, B. 1974. Genetic recombination in Rhodopseudomonas capsulatus. Proc. Natl. Acad. Sci. USA 71:971–973.

77. Marrs, B. 1981. Mobilization of the genes for photosynthesis from Rhodopseudomonas capsulatus by a promiscuous plasmid. J. Bacteriol. 140:1003–1012.

78. Meinhardt, S. W., P. J. Kiley, S. Kaplan, A. R. Crofts, and S. Harayama. 1985. Characterization of light-harvesting mutants of Rhodopseudomonas sphaeroides. 1. Measurement of the efficiency of energy transfer from light-harvesting complexes to the reaction center. Arch. Biochem. Biophys. 236:130–139.

79. Meyer, T. E., and M. D. Kamen. 1982. New perspectives on c-type cytochromes. Adv. Protein Chem. 35:105–212.

80. Michel, H., O. Epp., and J. Deisenhofer. 1986. Pigment-protein interactions in the photosynthetic reaction centre from Rhodopseudomonas viridis. EMBO J. 5:2445–2451.

81. Miller, L., and S. Kaplan. 1978. Plasmid transfer and expression in Rhodopseudomonas sphaeroides. Arch. Biochem. Biophys. 187:229–234.

82. Monger, T. G., and W. W. Parson. 1977. Single-triplet fusion in Rhodopseudomonas sphaeroides chromatophores: a probe of the organization of the photosynthetic apparatus. Biochem. Biophys. Acta 406:393–407.

83. Muller, E. D., J. Chory, and S. Kaplan. 1985. Cloning and characterization of the gene product of the form II ribulose-1,5-bisphosphate carboxylase gene of Rhodopseudomonas sphaeroides. J. Bacteriol. 161:469–472.

84. Mundo, F. E., and S. Kaplan. 1984. Plasmid rearrangements in the photosynthetic bacterium Rhodopseudomonas sphaeroides. J. Bacteriol. 158:1094–1103.

85. Mundo, F. E., W. D. Shepherd, M. M. Watkins, S. A. Kuhl, and S. Kaplan. 1985. Broad-host range plasmid vector for the in vitro construction of transcriptional/ translational fusions. J. Bacteriol. 169:3219–3276.

86. Nolte, J. 1983. Control of the formation of bacteriochlorophyll B875- and B850-bacteriochlorophyll complexes in Rhodo-
mutagenesis in Gram-negative bacteria. Bio/Technology 1:37–45.
96. Sistrom, W. R. 1975. List of mutant strains, p. 927–934. In R. K. Clayton and W. R. Sistrom (ed.). The photosynthetic
bacteria. Plenum Publishing Corp., New York.
97. Sistrom, W. R., A. Macalusa, and R. Pledger. 1984. Mutants of
Rhodospseudomonas sphaeroides useful in genetic analysis.
Arch. Microbiol. 138:161–165.
98. Snozzi, M., and A. R. Crofts. 1984. Electron transport in
chromatophores from Rhodospseudomonas sphaeroides Ga
fused with liposomes. Biochim. Biophys. Acta 766:451–463.
99. St. G. Bowen, A. R., and J. M. Pemberton. 1985. Mapping of
the genome of Rhodospseudomonas sphaeroides. p. 885.
In D. R. Helinski, S. N. Cohen, D. B. Clewell, D. A. Jackson,
and A. Hollaender (ed.). Plasmids. Plenum Publishing Corp.,
New York.
100. Stark, W. F., J. A. and K. Muehlethaler. 1986. Localisation
of reaction centre and light harvesting complexes in the
photosynthetic unit of Rhodospseudomonas viridis. Arch.
Microbiol. 146:130–133.
101. Tadros, M. H., R. Frank, and G. Drews. 1986. Localization of
the exposed N-terminal region of the B800-850-a and β
light-harvesting polypeptides on the cytoplasmic surface of
Rhodospseudomonas capsulata chromatophores. J. Bacteriol.
167:79–100.
102. Tai, S.-P., J. H. Hager, and S. Kaplan. 1986. Phospholipid
transfer activity in synchronous populations of Rhodobacter
sphaeroides. Biochim. Biophys. Acta 859:198–208.
103. Tai, S.-P. and S. Kaplan. 1984. Purification and properties of
a phospholipid transfer protein from Rhodospseudomonas
sphaeroides. J. Biol. Chem. 259:12178–12183.
104. Takemoto, J., and J. Lascelles. 1973. Coupling between bacte-
riochlorophyll and membrane protein synthesis in Rhodospseu-
domonas sphaeroides. Proc. Natl. Acad. Sci. USA 70:799–803.
105. Taylor, D. P., S. N. Cohen, W. G. Clark, and B. L. Marrs.
1983. Alignment of genetic and restriction maps of the photosyn-
synthesis region of the Rhodospseudomonas capsulata chro-
mosome by a conjugation-mediated marker rescue technique. J.
Bacteriol. 154:580–590.
106. Theiler, R., F. Suter, J. D. Piennoyer, H. Zuber, and R. A.
Niederman. 1985. Complete amino acid sequence of the B875
light-harvesting protein of Rhodospseudomonas sphaeroides
strain 2.4.1. FEBS Lett. 184:231–236.
107. Theiler, R., F. Suter, V. Wiemken, and H. Zuber. 1984. The
light-harvesting polypeptides of Rhodospseudomonas
sphaeroides R-26:1. I. Isolation, purification and sequence analysis.
Hoppe-Seyler’s Z. Physiol. Chem. 365:703–719.
108. Theiler, R., F. Suter, H. Zuber, and R. J. Cogdell. 1984. A
comparison of the primary structures of the two B800-850-
apoproteins from wild-type Rhodospseudomonas sphaeroides
strain 2.4.1 and a carotenoidless mutant strain R26.1. FEBS
Lett. 175:231–237.
109. Tybulewicz, V. L. J., G. Falk, and J. E. Walker. 1984.
Rhodospseudomonas brasicae atp operon:nucleotide sequence
and transcription. J. Mol. Biol. 179:201–215.
110. Weaver, K. E., and R. B. Tabita. 1985. Complementation of a
Rhodospseudomonas sphaeroides ribulose bisphosphate car-
boxylase/oxygenase regulatory mutant from a genomic library.
J. Bacteriol. 164:147–154.
111. Wette, W., T. Wacker, M. Leis, W. Kreutz, J. Shiozawa,
N. Gadon, and G. Drews. 1985. Crystallization of the photosyn-
thetic light-harvesting pigment-protein complex B800-850 of
Rhodospseudomonas capsulata. FEBS Lett. 182:265–267.
112. Wheeler, K. A., W. Schäfer, F. Lottspeich, and H. Michel. 1987.
The cytochrome subunit of the photosynthetic reaction center
from Rhodospseudomonas viridis is a lipoprotein. Biochemistry
26:2909–2914.
113. Williams, J. C., L. A. Steiner, and G. Feher. 1986. Primary
structure of the reaction center from Rhodospseudomonas
sphaeroides Biochim. Biophys. Acta 764:1–17.
114. Williams, J. C., L. A. Steiner, G. Feher, and M. I. Simon. 1984.
Primary structure of the L subunit of the reaction center of
pseudomonas sphaeroides mutant strain H5. Arch. Microbiol.
136:312–316.
87. Parson, W. W., and D. Holten. 1986. Primary electron transfer
reactions in photosynthetic bacteria: energetics and kinetics of
transient states, p. 338–343. In L. A. Stuehelin and C. J.
Arntzen (ed.). Photosynthesis III: photosynthetic membranes.
Encyclopedia of plant physiology, new series, vol. 19. Springer-
Verlag, New York.
Rhodopseudomonas sphaeroides. Proc. Natl. Acad. Sci. USA 81:3703–3708.

124. Williams, J. C., L. A. Steiner, R. C. Odgen, M. I. Simon, and G. Feher. 1983. Primary structure of the M subunit of the reaction center from Rhodopseudomonas sphaeroides. Proc. Natl. Acad. Sci. USA 80:6505–6509.

125. Willison, J. C., G. Ahumbo, J. Chabert, J.-P. Magnin, and P. M. Vignais. 1985. Genetic mapping of the Rhodopseudomonas capsulata chromosome shows nonclustering of genes involved in nitrogen fixation. J. Gen. Microbiol. 131:3001–3015.

126. Winans, S. C., P. R. Ebert, S. E. Stachel, M. P. Gordon, and E. W. Nester. 1986. A gene family essential for Agrobacterium virulence is homologous to a family of positive regulatory loci. Proc. Natl. Acad. Sci. USA 83:8278–8282.

127. Wraight, C. A., D. R. Leuking, R. T. Fraley, and S. Kaplan. 1978. Synthesis of photopigments and electron transport components in synchronous phototrophic cultures of Rhodopseudomonas sphaeroides. J. Biol. Chem. 253:465–471.

128. Yeates, T. O., H. Komiyama, D. C. Rees, J. P. Allen, and G. Feher. 1987. Structure of the reaction center from Rhodobacter sphaeroides R-26: membrane-protein interactions. Proc. Natl. Acad. Sci. USA 84:6438–6442.

129. Yen, G. S. I., B. D. Cain, and S. Kaplan. 1984. Cell-cycle specific biosynthesis of the photosynthetic membrane of Rhodopseudomonas sphaeroides: structural implications. Biochim. Biophys. Acta 777:41–45.

130. Yen, H.-C., and B. Marrs. 1976. Maps of genes for carotenoid and bacteriochlorophyll biosynthesis in Rhodopseudomonas capsulata. J. Bacteriol. 126:619–629.

131. Yen, H.-C., and B. L. Marrs. 1977. Growth of Rhodopseudomonas capsulata under anaerobic dark conditions with dimethylsulfoxide. Arch. Biochem. Biophys. 181:411–418.

132. Youvan, D. C., E. J. Bylina, A. M. Alberti, H. Begusch, and J. E. Hearst. 1984. Nucleotide and deduced polypeptide sequences of the photosynthetic reaction center. B870 antenna and flanking sequences from R. capsulata. Cell 37:949–957.

133. Youvan, D. C., J. E. Hearst, and B. L. Marrs. 1983. Isolation and characterization of enhanced fluorescence mutants of Rhodopseudomonas capsulata. J. Bacteriol. 154:748–755.

134. Youvan, D. C., and S. Ismail. 1985. Light-harvesting II (B800-850 complex) structural genes from Rhodopseudomonas capsulata. Proc. Natl. Acad. Sci. USA 82:58–62.

135. Youvan, D. C., S. Ismail, and E. J. Bylina. 1985. Chromosomal deletion and plasmid complementation of the photosynthetic reaction center and light-harvesting genes from Rhodopseudomonas capsulata. Gene 38:19–30.

136. Youvan, D. C., and B. L. Marrs. 1984. Molecular genetics and the light reactions of photosynthesis. Cell 39:1–3.

137. Zhu, Y. S., D. W. Cook, F. Leach, G. A. Armstrong, M. Alberti, and J. E. Hearst. 1986. Oxygen-regulated mRNAs for light-harvesting and reaction center complexes and for bacteriochlorophyll and carotenoid biosynthesis in Rhodobacter capsulatus during the shift from anaerobic to aerobic growth. J. Bacteriol. 168:1180–1188.

138. Zhu, Y. S., and J. Hearst. 1986. Regulation of expression of genes for light-harvesting antenna proteins LH1 and LHII; reaction center polypeptides RC-L, RC-M, and RC-H; and enzymes of bacteriochlorophyll and carotenoid biosynthesis in Rhodobacter capsulatus by light and oxygen. Proc. Natl. Acad. Sci. USA 83:7613–7616.

139. Zhu, Y. S., and S. Kaplan. 1985. Effects of light, oxygen, and substrates on steady-state levels of mRNA coding for ribulose 1,5-bisphosphate carboxylase and light-harvesting reaction center polypeptides in Rhodopseudomonas sphaeroides. J. Bacteriol. 162:925–932.

140. Zhu, Y. S., P. J. Kiley, T. J. Donohue, and S. Kaplan. 1986. Origin of the mRNA stoichiometry of the puf operon in Rhodobacter sphaeroides. J. Biol. Chem. 261:10366–10374.

141. Zuber, H. 1985. Structure and function of light-harvesting complexes and their polypeptides. Photochem. Photobiol. 42:821–844.

142. Zuber, H. 1986. Structure of light-harvesting antenna complexes of photosynthetic bacteria, cyanobacteria and red algae. Trends Biochem. Sci. 11:414–419.

143. Zuber, H. 1986. Primary structure and function of the light-harvesting polypeptides from cyanobacteria, red algae, and purple photosynthetic bacteria. p. 238–251. In L. A. Stuehelin and C. J. Arntzen (ed.). Photosynthesis III: photosynthetic membranes. Encyclopedia of plant physiology, new series, vol. 19. Springer-Verlag, New York.