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Genetic–Physical Mapping of a Photosynthetic Gene Cluster from R. capsulata

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Summary

Transposon mutagenesis and complementation analysis of the photosynthesis genes in Rhodopseudomonas capsulata is presented utilizing Tn5.7 mutagenized R-primes. The R-prime pRPS404 contains many of the genes necessary for the differentiation of the photosynthetic apparatus. Utilizing homologous recombination, 30 independent copies of Tn5.7 were inserted into the R. capsulata chromosome with subsequent deletion of wild-type alleles. Mutants were characterized by absorption spectroscopy, SDS-PAGE, and determination of capability for photosynthetic growth. Many mutations in the bacteriochlorophyll and carotenoid biosynthetic pathways were isolated. A regulatory mutation was isolated affecting reaction-center synthesis as well as a 44 kd heme-containing polypeptide. Complementation analysis using various pRPR404::Tn5.7 plasmids has led to the postulation of transcriptional units.

Introduction

Rhodopseudomonas capsulata is a gram-negative facultative anaerobic bacterium with a metabolic versatility that allows growth under a variety of conditions, including photosynthesis and chemoheterotrophy. During low pO_2, an intracytoplasmic membrane system is synthesized, which is capable of light-driven cyclic electron transport akin to cyclic electron transport involving photosystem I of higher plants. At least three distinct types of pigment–protein complexes are synthesized and integrated into the intracytoplasmic membrane under photosynthetic growth conditions: two light-harvesting complexes, LHI and LHII, and a reaction-center complex, where primary photochemistry occurs. The two light-harvesting complexes function to increase the cross-sectional area for absorption of light. They transfer energy to the reaction center where electrons from bacteriochlorophyll are used to generate a transmembrane proton gradient. The electrons are cycled back to the reaction center after passage through the cytochrome b-c_1 oxidoreductase complex and cytochrome c_2 (Cramer and Crofts, 1982). The proton gradient is utilized to generate ATP via coupling factor. Because of the metabolic versatility, the Rhodopseudomonads offer a model system for the analysis of photosynthesis genes. Unlike higher plants, photosynthetic mutants are viable. During aerobic culture the photosynthetic apparatus is repressed, but cells can be grown anaerobically in the dark using DMSO as a terminal electron acceptor (Yen and Marrs, 1977) by inducing a cytochrome c_{555} and a DMSO reductase (Paul Weaver, personal communication). Under these growth conditions cells fully induce their photosynthetic apparatus even though energy is derived from carbohydrate.

Study of photosynthesis genes in R. capsulata is aided by an indigenous gene-transfer agent (Yen and Marrs, 1976), isolation of a conjugative R-prime plasmid, pRPS404, containing R. capsulata DNA, which complements many mutations affecting the differentiation of the photosynthetic apparatus (Marrs, 1981), and conjugation-mediated marker-rescue techniques utilizing mobilization of pBR322 derivatives containing subclones of the R-prime (Taylor et al., 1983). The genes on the R-prime plasmid pRPS404 have been probed by all of the above techniques. A partial genetic–physical map exists for the photosynthetic gene cluster contained on pRPS404 (Taylor et al., 1983). Recently, the structural genes for the reaction-center and LHI complexes have been sequenced (Youvan et al., 1984).

In this report we extend the genetic–physical map of this region by the use of transposon Tn5.7 mutagenesis and complementation analysis with various pRPS404::Tn5.7 plasmids. Transposon Tn5.7 is 7.6 kb and contains the IS50 elements from Tn5 and the antibiotic resistance markers from Tn7 (Zsebo et al., 1984). Recently, a gene-replacement system has been described, utilizing homologous recombination and the inherent instability of the R-prime in a *rec*$^+$ background (Youvan et al., 1982). This instability arises from IS21-mediated intramolecular recombination. The gene-dosage effect of streptomycin (Sm) resistance, provided by Tn5.7, and the instability of the R-prime have been utilized to isolate mutations in previously undescribed genetic loci effecting the differentiation of intracytoplasmic photosynthetic structures. We have isolated 45 independent insertions of Tn5.7 into the R. capsulata DNA contained in pRPS404, 30 of which have been recombined into the chromosome of R. capsulata. Southern analysis of the R. capsulata mutants confirms the location of the transposon to be identical with the insertion site in the R-prime in E. coli.

Mutations in either the bacteriochlorophyll or carotenoid biosynthetic pathway are easily identified by an alteration in colony pigmentation. Mutations effecting energy transfer result in increased fluorescence, and colony fluorescence was monitored by infra-red photography (Youvan et al., 1983). Phenotypic characterization of photosynthetic mutants included determining capability for photosynthetic growth (PS+/−), absorption spectroscopy, and SDS-PAGE of subcellular chromatophores. Chromatophores are membrane vesicles originally continuous with the cytoplasmic membrane that contain the photosynthetic pigment–protein complexes. New phenotypic classes were identified including regulatory mutations affecting reaction centers as well as previously unidentified loci involved with

bacteriochlorophyll and carotenoid biosynthesis. Complementation analysis has tentatively identified transcriptional operons for certain photopigment genes.

Results and Discussion

Tn5.7 Mutagenesis and Gene Replacement

To identify photosynthesis genes located on the R-prime plasmid pRPS404, 45 insertions of Tn5.7 were isolated as previously described (Zsebo et al., 1984). The location of the transposon was determined by restriction enzyme digests of various pRPS404::Tn5.7 plasmids using Xho I and Bgl II (data not shown). The transposon insertions were named by their distance in kilobases counter-clockwise from the Eco RI restriction site at 0 kb on pRPS404, Figure 1. These transposon-containing plasmids were conjugated from E. coli into R. capsulata by selection for the transposon-mediated resistance to Sm. Continuous repurification on Sm selects for the recombination of Tn5.7 into the chromosome since the R-prime has been shown to be unstable in *rec+* strains (Youvan et al., 1982). Therefore, stable resistance to Sm can arise from either double-recombination events between the chromosome and the plasmid, placing the transposon in the chromosome and the wild-type gene on the plasmid, a single recombination event integrating the entire plasmid into the chromosome, or a transposition into the chromosome. The majority of events that were observed by Southern analysis were double-recombination events, replacing the wild-type gene with the transposon insertion. Since there is 50 kb of homologous DNA between the pRPS404::Tn5.7 plasmids and the chromosome, we expect this to be the major event. On occasion, we have observed deletions of 30 kb or more in the R. capsulata DNA due to transpositional insertion, but only in cases where the transposon inserted into the Bam HI I fragment. This fragment is thought to contain a promoter for the *bchE* gene (Taylor et al., 1983), which may make insertions unstable. The two strains in which we have observed deletions are KZR3H2 and KZR8A8, with original Tn5.7 insertions at 51.9 and 50.8 kb, respectively. Any integration of the plasmid with subsequent deletion of flanking sequences is readily detected by probing the genomic DNA with pRPS404 in a Southern hybridization experiment. If the transposon is placed in the chromosome by a double-recombination event, the wild-type gene is lost since there is no longer any selection pressure for maintenance of an intact plasmid.

In order to confirm that the mutant phenotypes observed were due to homologous recombination of the fragment that contains the transposon into the chromosome, the DNA of all mutant strains was examined by Southern hybridization technique (Smith and Summers, 1980). Figure 2 is an example of a genomic Southern analysis of Tn5.7-generated mutants of R. capsulata. DNA isolated from each strain was digested with Eco RI and probed with ^{32}P-labeled Tn5.7 probe pRS5 (A) (Lichtenstein and Brenner, 1981); a vector probe pLM2 (B) (Marrs, 1981), or pRPS404 (C). The bands that hybridize to the pRPS404 probe are a combination of native R. capsulata genomic DNA fragments and remnants of deleted R-primes that are homologous to the vector portion of pRPS404. The junction fragments between the R. capsulata DNA in pRPS404 and the genome are observed in an Eco RI digest. These fragments are maintained in the Tn5.7-generated mutants, indicating that there were no perturbations to the flanking DNA. It has been found that deletion events involving the

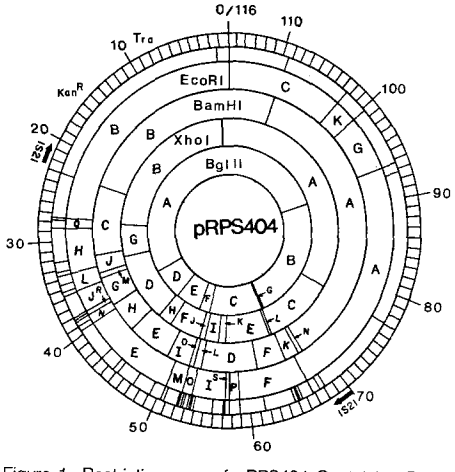

Figure 1. Restriction map of pRPS404 Containing Restriction Fragments Discussed in the Text

The R. capsulata DNA is bracketed by IS21 elements and is between ~23 and 68 kb.

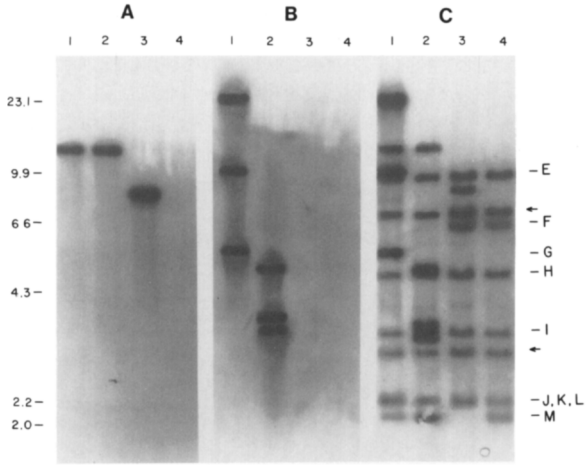

Figure 2. Southern Analysis of Tn5.7 Insertions into Photosynthesis Genes of R. capsulata

Total DNA prepared from R. capsulata strains that received different pRPS404::Tn5.7 plasmids was subject to digestion with Eco RI, electrophoresed in duplicate in a 0.7% agarose gel, and transferred bidirectionally to nitrocellulose. One filter was hybridized to a transposon probe, pRS5 (A); another to a vector probe, pLM2 (B); and a third to pRPS404 (C). Molecular weights were determined from coelectrophoresis of end-labeled λ/*Hind* III fragments. Letters on the right refer to Eco RI fragments from the plasmid pRPR404. Through historical circumstance, there is no Eco RI D fragment. The fragments joining R. capsulata DNA from pRPS404 and the chromosome are 8.5 and 3.4 kb and are marked by an arrow. Lane 1, F1696Ω63.6; lane 2, *rxcD*Ω65.7; lane 3, Ω51.7; and lane 4, SB1003, wild type.

R-prime occur via intramolecular recombination between the IS21 elements but that additional deletion events occur extending into the Km^R gene and into *sex*, the locus involved with surface exclusion in RP1-type plasmids (Hedges et al., 1976). Similar phenomena have been observed with spontaneous deletions of R68.45, a related plasmid, in Pseudomonas aeruginosa (Haas and Riess, 1983) and Erwinia carotorora (Currier and Morgan, 1982). Deletions of the Km^R gene were found, as well as deletions of the transfer region, *tra*. If gene replacement has gone to completion, then the Tn5.7 target restriction fragment should be missing from the hybridization pattern and be replaced by a hybrid band with the molecular weight of the transposon, 7.6 kb, plus the original fragment. As is illustrated in Figure 2C, when compared to the hybridization pattern from wild type, mutant strains lack only one fragment, which corresponds to the fragment mutated by Tn5.7 (for example, Ω 51.7 in the Eco RI M fragment). This band is missing in Figure 2C, lane 3. In addition, there are new bands that either hybridize to the transposon probe (A) or to the vector probe (B). These bands result from partial deletions of pRPS404 extending into various regions of the R-factor.

Phenotypic Characterization of Tn5.7-Generated Mutants

Once pRPS404::Tn5.7 plasmids were conjugated into R. capsulata, many transconjugants showed abnormal pigmentation or fluorescence (Youvan et al., 1983) after several repurifications on Sm. These phenotypes were observed as sectors in colonies, visible only as the wild-type alleles were deleted. Analysis of mutant phenotypes included determination of capability for photosynthetic growth, PS+/−, visible absorption spectroscopy of chromatophore fractions, and SDS-PAGE (see Experimental Procedures). PS+/− phenotypes are listed in Table 1. Absorption spectra of photosynthetic membrane fractions are mostly indicative of LHI and LHII since reaction centers are a minor component. The LHI complex absorbs maximally at 870 nm and LHII absorbs at 800 and 850 nm (Lien et al., 1973). Carotenoids absorb between 400–500 nm (Scolnik et al., 1980). Bacteriochlorophyll absorption peaks common to all complexes are at 590 and 375 nm (Jensen et al., 1964). Figure 3 is an absorption spectrum of wild-type R. capsulata, SB1003, and two mutants resulting from insertions 28.0, K2R8G2, and 39.8, K2R9F4. Insertion 28.0 results in a *bchA* phenotype, which is accumulation of 2-evinyl,2-hydroxyethyl chlorophyllide *a* and chlorophyllide *a*, both bacteriochlorophyll precursors that absorb at around 665 nm (Biel and Marrs, 1983). When mature bacteriochlorophyll is not made, the polypeptides of the various complexes never appear in the membrane fraction. As can be seen in Figure 3, insertion 28.0, strain K2R8G2, results in a lack of absorption peaks attributable to LHI and LHII.

Sequence analysis of the Bam HI F fragment has identified the structural gene for the H subunit of the reaction center, *rxcB*, (Youvan et al., 1984) and two open reading frames possibly coding for a 77 amino acid hydrophilic and a 477 amino acid hydrophobic protein. Tn5.7 insertion 61.6 maps within the 77 amino acid open reading frame and results in a bacteriochlorophyll$^-$ phenotype. This 77 amino acid open reading frame has been designated as the *bchL* locus. Insertion 61.6 is not bacteriochlorophyll$^-$ because of polar inactivation of a *bch* gene transcriptionally downstream from the open reading frame because transcription is towards the 477 amino acid open reading frame, F1696, where insertions alter LHI and reaction centers but do not effect bacteriochlorophyll synthesis.

The identification of the *bchI, bchJ,* and *bchK* loci is based on the generation of bacteriochlorophyll$^-$ phenotypes by insertions that complement flanking *bch* genes. Detailed discussion of these loci is found below in the section on complementation analysis. These are tentative identifications of new genetic loci but the simplest interpretation is that these loci are distinct from previously isolated genes. It has not been determined what compounds are accumulated by these mutants, if any, but the absorption maxima in methanol extracts are listed in Table 1. Many of the bacteriochlorophyll mutants accumulate more than one compound. Possibly, some bacteriochlorophyll biosynthetic enzymes have more than one substrate. Alternatively, membrane-bound enzyme complexes exist that may be fully active only when all components of the complex are present. These *bch* mutants should be helpful in further elucidation of the bacteriochlorophyll biosynthetic pathway, part of which is already known (Biel and Marrs, 1983).

Insertions altering carotenoid formation but complementing existing *crt* genes were isolated and designated *crtI* and *crtJ*. Mutation *crtI*Ω39.8 results in a blue-green phenotype, which is predominantly associated with a block in carotenoid biosynthesis at an early stage but also demonstrates a pleiotropic effect on LHII; the bacteriochlorophyll-binding LHII polypeptides are made but no absorption from LHII is observed. Blue-green mutants make normal amounts of bacteriochlorophyll and are PS^+ (Yen and Marrs, 1976). Only the nonbacteriochlorophyll-binding (Cogdell and Crofts, 1978) 14 kd LHII polypeptide is missing in blue-green mutants (Figures 4f, 4g, and 4h. As seen in Figure 3, mutation *crtI*Ω39.8, strain KZR9F4, results in an absorption spectrum devoid of carotenoid and LHII absorption peaks even though the 8 and 10 kd LHII polypeptides are present. Possibly, the 14 kd LHII polypeptide stabilizes the bacteriochlorophyll bound to the 8 and 10 kd polypeptides. The *crtI* and *crtJ* genes identified by insertions of Tn5.7 both are blocked in early stages of carotenoid biosynthesis and accumulate no visibly absorbing carotenoids.

Insertions of Tn5.7 into the structural genes of the reaction-center subunits have been isolated. Reaction-center mutations are pleiotropic in that if one subunit of the reaction center is not made, none of the subunits are integrated into the membrane. The SDS-PAGE profiles of mutants *rxcA*Ω26.3, F1696Ω63.5, F1696Ω63.6, F1696Ω64.4, and *rxcD*Ω65.7 are shown in Figures 4a, 4b, 4c, 4d, and 4e, respectively. Insertion 26.3 is located in

Table 1. Bacterial Strain Table

Genotype	Phenotype[a], Comments, and References
E. coli	
NECO100 *leu⁻thr⁻thi⁻trp⁻recA⁻lacY⁻*	Rif^R, Youvan, et al., 1982
HB101 *pro⁻leu⁻thr⁻lacY⁻hsdM⁻hsdR⁻recA⁻*	Sm^R, S. Cohen
NECO205 HB101[pLAFR1]	Friedman et al., 1982
NECO200 HB101[pRK2013]	Ditta et al., 1980
KZE8E9 NECO100[pRPS404Ω(26.3 kb::Tn5.7)]	this work
KZE8G2 NECO100[pRPS404Ω(28.0 kb::Tn5.7)]	this work
KZEIA4 NECO100[pRPS404Ω(28.9 kb::Tn5.7)]	this work
KZE8H12 NECO100[pRPS404Ω(29.5 kb::Tn5.7)]	this work
KZE8D6 NECO100[pRPS404Ω(30.3 kb::Tn5.7)]	this work
KZE9C4 NECO100[pRPS404Ω(31.2 kb::Tn5.7)]	this work
KZE3G7 NECO100[pRPS404Ω(31.4 kb::Tn5.7)]	this work
KZE8B8 NECO100[pRPS404Ω(33.3 kb::Tn5.7)]	this work
KZE8F2 NECO100[pRPS404Ω(33.4 kb::Tn5.7)]	this work
KZE3H3 NECO100[pRPS404Ω(33.7 kb::Tn5.7)]	this work
KZE8F6 NECO100[pRPS404Ω(33.8 kb::Tn5.7)]	this work
KZE8A1 NECO100[pRPS404Ω(34.6 kb::Tn5.7)]	this work
KZE9F12 NECO100[pRPS404Ω(36.4 kb::Tn5.7)]	this work
KZE9D10 NECO100[pRPS404Ω(36.8 kb::Tn5.7)]	this work
KZE9F4 NECO100[pRPS404Ω(39.8 kb::Tn5.7)]	this work
KZE8B3 NECO100[pRPS404Ω(40.2 kb::Tn5.7)]	this work
KZE9A12 NECO100[pRPS404Ω(41.0 kb::Tn5.7)]	this work
KZE8D7 NECO100[pRPS404Ω(41.6 kb::Tn5.7)]	this work
KZEIA7 NECO100[pRPS404Ω(43.1 kb::Tn5.7)]	this work
KZE8E12 NECO100[pRPS404Ω(43.2 kb::Tn5.7)]	this work
KZE9A6 NECO100[pRPS404Ω(44.6 kb::Tn5.7)]	this work
KZE9D5 NECO100[pRPS404Ω(46.9 kb::Tn5.7)]	this work
KZE9C10 NECO100[pRPS404Ω(47.4 kb::Tn5.7)]	this work
KZE3A1 NECO100[pRPS404Ω(49.2 kb::Tn5.7)]	this work
KZE8G5 NECO100[pRPS404Ω(49.8 kb::Tn5.7)]	this work
KZR8A8 NECO100[pRPS404Ω(50.8 kb::Tn5.7)]	this work
KZR9A4 NECO100[pRPS404Ω(51.0 kb::Tn5.7)]	this work
KZE8F1 NECO100[pRPS404Ω(51.7 kb::Tn5.7)]	this work
KZE3H2 NECO100[pRPS404Ω(51.9 kb::Tn5.7)]	this work
KZE9E3 NECO100[pRPS404Ω(53.5A kb::Tn5.7)]	this work
KZE8D9 NECO100[pRPS404Ω(53.5B kb::Tn5.7)]	this work
KZE9E11 NECO100[pRPS404Ω(55.0 kb::Tn5.7)]	this work
KZE8G9 NECO100[pRPS404Ω(55.1 kb::Tn5.7)]	this work
KZE9G5 NECO100[pRPS404Ω(56.7 kb::Tn5.7)]	this work
KZE8H10 NECO100[pRPS404Ω(58.0 kb::Tn5.7)]	this work
KZE9F2 NECO100[pRPS404Ω(58.5 kb::Tn5.7)]	this work
KZE8G12 NECO100[pRPS404Ω(59.7 kb::Tn5.7)]	this work
KZE8B4 NECO100[pRPS404Ω(59.8 kb::Tn5.7)]	this work
KZE9C6 NECO100[pRPS404Ω(60.0 kb::Tn5.7)]	this work
KZE8A9 NECO100[pRPS404Ω(61.6 kb::Tn5.7)]	this work
KZE8B1 NECO100[pRPS404Ω(63.5 kb::Tn5.7)]	this work
KZE9F6 NECO100[pRPS404Ω(63.6 kb::Tn5.7)]	this work
KZE3A3 NECO100[pRPS404Ω(64.4 kb::Tn5.7)]	this work
KZE9C1 NECO100[pRPS404Ω(65.6 kb::Tn5.7)]	this work
KZEIA1 NECO100[pRPS404Ω(65.7 kb::Tn5.7)]	this work
KZE9B4 NECO100[pRPS404Ω(67.9 kb::Tn5.7)]	this work
R. capsulata	
KZR8E9 *rxcA*Ω(26.3::Tn5.7)*rif-10crtD223*	PS$^-$, RC$^-$, LHII^{red}, Sm^R, Sp^R, Km^R, this work
KZR8G2 *bchA*Ω(28.0::Tn5.7)*rif-10crtD223*	PS$^-$, P670, Sm^R, Sp^R, Km^R, this work
KZRIA4 *bchA*Ω(28.9::Tn5.7)*rif-10crtD223*	PS$^-$, P670, Sm^R, Sp^R, Km^R, this work
KZR8H12*bchA*Ω(29.5::Tn5.7)*rif-10crtD223*	PS$^-$, P670, Sm^R, Sp^R, Km^R, this work
KZR8D6 *bchA*Ω(30.3::Tn5.7)*rif-10crtD223*	PS$^-$, P670, Sm^R, Sp^R, Km^R, this work
KZR9C4 *bchA*Ω(31.2::Tn5.7)*rif-10crtD223*	PS$^-$, P670, Sm^R, Sp^R, Km^R, this work
KZR3G7 *bchA*Ω(31.4::Tn5.7)*rif-10crtD223*	PS$^-$, P670, Sm^R, Sp^R, Km^R, this work
KZR8A1 *crtE*Ω(34.6::Tn5.7)*rif-10*	PS$^+$, blue-green, Sm^R, Sp^R, Km^R, this work
KZR9F4 *crtI*Ω(39.8::Tn5.7)*rif-10*	PS$^+$, blue-green, Sm^R, Sp^R, Km^R, this work
KZR9A12*crtA*Ω(41.0::Tn5.7)*rif-10*	PS$^+$, yellow, Sm^R, Sp^R, Km^R, this work
KZR8D7 *bchI*Ω(41.6::Tn5.7)*rif-10crtD223*	PS$^-$, trace P590, Sm^R, Sp^R, Km^R, this work
KZRIA7 *bchD*Ω(43.1::Tn5.7)*rif-10crtD223*	PS$^-$, Bchl$^-$, Sm^R, Sp^R, Km^R, this work
KZR8E12*bchD*Ω(43.2::Tn5.7)*rif-10crtD223*	PS$^-$, Bchl$^-$, Sm^R, Sp^R, Km^R, this work

Table 1. (Continued)

Genotype	Phenotype[a], Comments, and References
KZR9D5 Ω(46.9::Tn5.7)*rif-10*	PS$^+$, Sm^R, Sp^R, Km^R, this work
KZR9C10 Ω(47.4::Tn5.7)*rif-10*	PS$^+$, Sm^R, Sp^R, Km^R, this work
KZR3A1 *bchJ*Ω(49.2::Tn5.7)*rif-10crtD223*	PS$^-$, P750, P630, P580, P530, Sm^R, Sp^R, Km^R, this work
KZR8A8 Δ100(50.8::Tn5.7)*rif-10*	PS$^-$, Crt$^-$, Sm^R, Sp^R, Km^R, this work
KZR9A4 *bchE*Ω(51.0::Tn5.7)*rif-10*	PS$^-$, P620, P570, P538, Sm^R, Sp^R, Km^R, this work
KZR8F1 Ω(51.7::Tn5.7)*rif-10*	PS$^+$, Sm^R, Sp^R, Km^R, this work
KZR3H2 Δ101(51.9::Tn5.7)*rif-10*	PS$^-$, Crt$^-$, Sm^R, Sp^R, Km^R, this work
KRZ9E3 *crtJ*Ω(53.5A::Tn5.7)*rif-10*	PS$^+$, blue-green, Sm^R, Sp^R, Km^R, this work
KZR8D9 Ω(53.5B::Tn5.7)*rif-10*	Bchl$^-$, Sm^R, Sp^R, KmR, this work
KZR8G9 *bchF*Ω(55.1::Tn5.7)*rif-10crtD223*	PS$^-$, P630, P590, P570, P520, Sm^R, Sp^R, Km^R, this work
KZR9G5 *bchK*Ω(56.7::Tn5.7)*rif-10*	PS$^-$, Bchl$^-$, Sm^R, Sp^R, Km^S, this work
KZR8H10 *bchK*Ω(58.0::Tn5.7)*rif-10*	PS^{red}, Bchl^{red}, Sm^R, Sp^R, Km^S, this work
KZR9F2 *bchH*Ω(58.5::Tn5.7)*rif-10crtD223*	PS$^-$, P630, Sm$^-$, Sp^R, Km^R, this work
KZR8G12*bchH*Ω(59.7::Tn5.7)*rif-10crtD223*	PS$^-$, Bchl$^-$, Sm^R, Sp^R, Km^R, this work
KZR8A9 *bchL*Ω(61.6::Tn5.7)*rif-10crtD223*	PS$^-$, P630, P590, P570, Sm^R, Sp^R, Km^R, this work
KZR8B1 F1696Ω(63.5::Tn5.7)*rif-10*	PS$^+$, LHI^{red}, Sm^R, Sp^R, Km^R, this work
KZR9F6 F1696Ω(63.6::Tn5.7)*rif-10*	PS^{red}, RC^{red}, LHI^{red}, Sm^R, Sp^R, Km^R, this work
KZR3A3 F1696Ω(64.4::Tn5.7)*rif-10*	PS^{red}, RC^{red}, LHI^{red}, Sm^R, Sp^R, Km^R this work
KZRIA1 *rxcD*Ω(65.7::Tn5.7)*rif-10*	PS$^-$, RC$^-$, LHI^{red}, Sm^R, Sp^R, Km^S, this work
SB1003 *rif-10*	Marrs, 1981
Y142 *rxcA142 str-2*	PS$^-$, RC$^-$, LHI$^-$, Sm^R, Marrs, 1982
BPY38 *bchA538crtF129*	PS$^-$, P670, Scolnik, 1980
MB1007 *bchC1007*	PS$^-$, P710, Taylor, 1983
BW604 *crtE6*	PS$^+$, blue-green, Yen and Marrs, 1976
BPY26 *crtE526crtF129*	PS$^+$, blue-green, B. Marrs
W4 *crtB4*	PS$^+$, blue-green, Yen and Marrs, 1976
BPY51 *crtB551 crtF129*	PS$^+$, blue-green, B. Marrs
B301 *crtA301rif-10*	PS$^+$, yellow, Yen and Marrs, 1976
MB1008 *bchD1008*	PS$^-$, trace Bchl, Taylor et al., 1983
BPY61 *bchD561crtF129*	PS$^-$, trace Bchl, Scolnik, et al., 1980
BPY27 *bchG527crtF129*	PS$^-$, P760, Marrs, 1981
BRP4 *bchE604crtF129hsd-1str-2*	PS$^-$, P590, Sm^R, Taylor et al., 1983
MB1003 *bchF1003*	PS$^-$, P730, Taylor et al., 1983
BRP50 *bchH650crtF129hsd-1str-2*	PS$^-$, Bchl$^-$, Sm^R, Taylor et al., 1983

KZE strains are E. coli containing a library of pRPS404::Tn5.7 plasmids named for the insertion site in pRPS404 numbering counter-clockwise from the Eco RI site at 0 kb. KZR strains are R. capsulata containing Tn5.7 in the chromosome. The Tn5.7 insertion name in pRPS404 has been conserved for the sake of clarity. An example of this nomenclature is as follows: *rxcD*Ω65.7 means that the insertion of Tn5.7 at 65.7kb in pRPS404 is either in or is transcriptionally upstream from *rxcD*.

[a] The phenotypes listed for the KZR strains are as follows: the absence of structural polypeptides is indicated where applicable in reference to RC, LHI, or LHII. Bacteriochlorophyll mutations are either Bchl$^-$, no precursors detected, or a number is listed after P, indicating the absorption maxima of the Bchl precursor in methanol extracts. The R. capsulata strains used in this study other than KZR strains have references sited that describe the nomenclature used for bacteriochlorophyll precursors.

the structural gene for the M subunit of the reaction center, *rxcA*, and therefore results in a reaction-center$^-$ phenotype. F1696 has been identified as an open reading frame extending for 477 amino acids and ending just before *rxcB*. The direction of transcription is from left to right in Figure 6. The three insertions in this region all reduce LHI (Figures 4b, 4c, and 4d), but differ with respect to both the amount of reaction centers incorporated into the membrane fractions and capability for photosynthetic growth. The F1696Ω63.5 mutant shows only a slightly lower amount of reaction centers incorporated as compared to wild type and grows at normal rates photosynthetically. The F1696Ω63.6 and F1696Ω64.4 mutants have barely detectable levels of reaction centers as judged by SDS-PAGE, repeated at least three times with different chromatophore preparations, and grow at about 50% of the wild-type rate photosynthetically. The function of F1696 has not been determined. The transcriptional organization of this region is discussed below.

The *rxcD*Ω65.7 mutation is regulatory since the insertion is not in any of the structural genes for the reaction center. It is pleiotropic since the reaction centers and a 44 kd heme-staining polypeptide are absent from the membrane. C-type cytochromes are detected in SDS-PAGE profiles because they have a covalently attached heme group that can be stained by 3,3′,5,5′-tetra methyl benzidine-H_2O_2 (Hoyer–Hansen, 1980). Figure 5 shows an SDS-PAGE profile of SB1003, grown under various conditions,

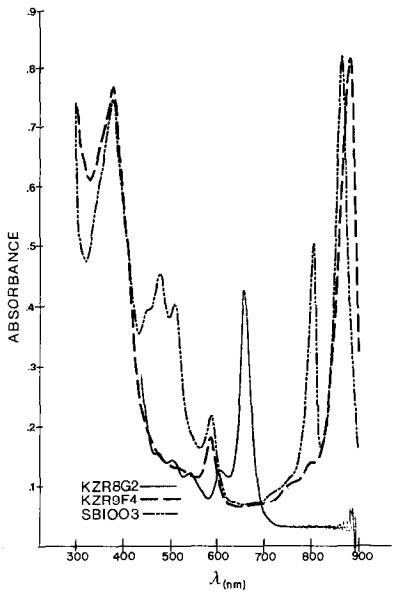

Figure 3. IR-Visible Absorption Spectroscopy of R. capsulata Strains

Chromatophore fractions from KZR8G2, containing Tn5.7 insertion 28.0; KZR9F4, containing Tn5.7 insertion 39.8; and SB1003, wild type; were isolated from cells cultured in RCV liquid plus DMSO, pyruvate, and glucose according to Experimental Procedures and subject to absorption spectroscopy.

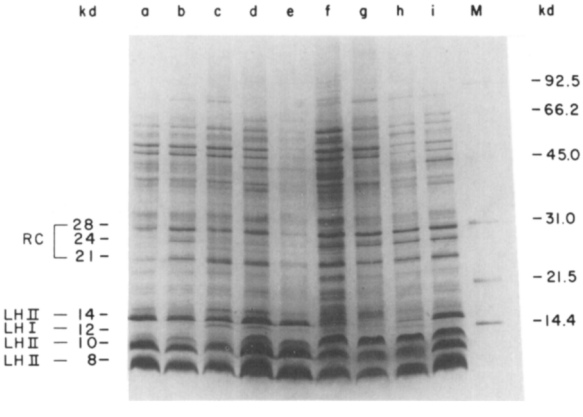

Figure 4. SDS-PAGE of Tn5.7-Generated Mutants

Chromatophore fractions were electrophoresed in a 9% to 18% acrylamide gradient gel according to Experimental Procedures and stained with Coomassie blue. Bands marked RC refer to the H, 28 kd; M, 24 kd; and L, 21 kd, subunits of the reaction center. The LHII 14, 10, and 8 kd polypeptides and LHI 12 kd polypeptide are indicated. Tn5.7-induced mutants are shown in lane a, *rxcA*Ω26.3; b, F1696Ω63.5; c, F1696Ω63.6; d, F1696Ω64.4; e, *rxcD*Ω65.7; f, *crtJ*Ω53.5A; g, *crtE*Ω34.6; h, *crtI*Ω39.8. Wild type, SB1003, is shown in lane i. Molecular weight standards are shown in lane M.

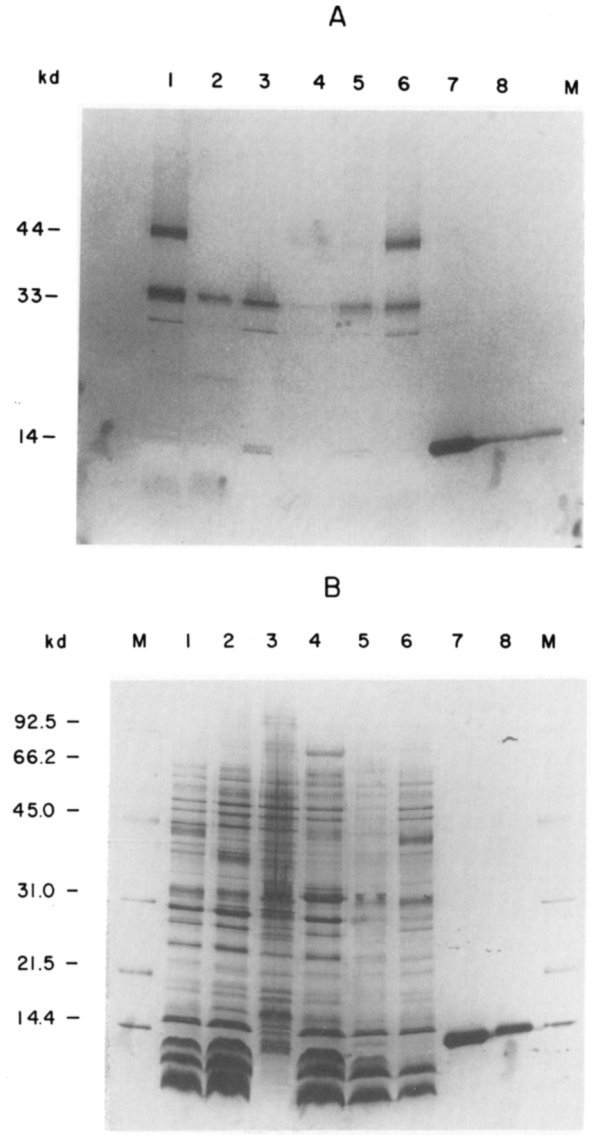

Figure 5. Comparison of Heme Content in Chromatophore Fractions Isolated from RC Mutants

SDS-PAGE was performed as in Figure 3. (A) shows the gel after heme-staining using 3,3′,5,5′-tetramethyl benzidine, and (B) shows the same gel after staining with Coomassie blue. Lane 1, SB1003 grown in the dark micro-aerophillically in the presence of DMSO; 2, SB1003 grown photosynthetically; 3, SB1003 grown aerobically; 4, SB1003, same as lane 1 but boiled for 5 min before loading; 5, *rxcD*Ω65.7; 6, *rxcA*Ω26.3; 7, cytochrome c′ (courtesy R. Salemne); 8, horse heart cytochrome c; M, molecular weight markers.

and two PS⁻ reaction-center mutants, *rxcA*Ω26.3 and *rxcD*Ω65.7. Figure 5A shows the heme-stained bands, and Figure 5B shows the gel after staining with Coomassie blue. The 44 kd band is only induced in wild type during dark micro-aerophilic growth the DMSO. However, this particular band is missing from the *rxcD*Ω65.7 mutant when grown with DMSO. The absence of this *c*-type cytochrome is not observed in reaction-center structural-gene mutations such as that caused by insertion *rxcA*Ω26.3 (Figure 5, lane 6). The 33 and 24 kd heme-staining polypeptides are probably part of the ubiquinone–cytochrome *b*-c_1 complex. The 14 kd heme-staining polypeptide is probably cytochrome c_2, which is loosely bound (Zannoni and Marrs, 1981) and is therefore easily washed off.

The 44 kd heme-staining polypeptide is only expressed under anaerobic conditions in the presence of DMSO. The pleiotropic nature of the *rxcD*Ω65.7 mutant is reminiscent of mutations in *fnr,* coding for a positive regulatory element

in E. coli (Shaw et al., 1983). It is a general regulatory protein that responds to the redox status of the cell and is necessary for the expression of a number of anaerobic respiratory systems. It is not known whether the RxcD protein acts at the level of transcription. If the block of reaction-center synthesis is at the level of transcription, then possibly the RxcD protein resembles the Fnr protein. If the block is post-transcriptional, then the RxcD protein may be involved in the insertion of reaction centers and the 44 kd heme-staining protein into the membrane. Another possible explanation of the pleiotropic nature of this mutation is that the 44 kd polypeptide is encoded by the Bam HI K fragment and is cotranscribed with the *rxcD* gene but the transposon insertion destabilizes the message so that neither protein is made. Further studies on the nature of the *rxcD*Ω65.7 mutation are in progress.

Certain insertions never result in abnormal colony sectors, even after many repurifications. Three of these strains, resulting from insertions 46.9, 47.4, and 51.7, appear to have a wild-type photosynthetic apparatus. The recombination and deletion steps have gone to completion with these insertions, as judged by Southern analysis (data not shown).

Complementation Analysis by Various pRPS404::Tn5.7 Plasmids

Complementation of carotenoid, bacteriochlorophyll, and reaction-center mutations with pRPS404::Tn5.7 plasmids was accomplished in order to facilitate genetic–physical mapping. Since the pRPS404::Tn5.7 plasmids are unstable upon continuous repurification, complementation has to be distinguished from recombination. This was accomplished in the *rec*$^+$ R. capsulata by the difference in frequency of complementation versus recombination-based marker rescue. Complementation of bacteriochlorophyll and reaction-center mutations was determined by capacity for photosynthetic growth, and carotenoid mutations by inspection for colored carotenoid production. Various pRPS404::Tn5.7 plasmids were transferred into R. capsulata strains harboring mutations in *bch* and *crt* genes. The transconjugants were replica-plated after serial dilution and tested for photosynthetic growth or colony pigmentation. Positive complementation results in 10^4 to 10^5 out of 10^6 cells capable of photosynthetic growth or pigmentation, while negative complementation results in 10^1 to 10^2 out of 10^6 cells capable of photosynthetic growth or pigmentation due to recombination. Point mutations were tested simply by conjugation of a pRPS404::Tn5.7 plasmid into the R. capsulata strain with selection for either Sm^R or Sp^R. Certain KZR strains became Km^S due to a deletion into the Km^R gene and into *sex* so these strains could be tested for complementation by selecting for Km^R in conjugation events with pRPS404::Tn5.7 plasmids. However, the majority of KZR strains remained Km^R after the R-prime had deleted. In order to test these Tn5.7-induced mutations by complementation, the remains of the R-prime were eliminated by mobilizing in an incompatible plasmid, pLAFR1 (Friedman et al., 1982), which is a derivative of pRK290 and is a member of the incompatibility group P-1, and is tetracycline (Tet)-resistant. The efficiency of transfer into strains that were Km^R was about two orders of magnitude lower than transfer into wild-type R. capsulata. Some KZR strains were not capable of receiving pLAFR1 at all. This surface exclusion of pLAFR1 by the deleted R-prime suggests that *sex* on RP1, RP4, RK2, and R68 is near the Km^R gene. Tn7 mutagenesis of RP4 suggests that the location of *sex* is in a 13 kb region containing the Km^R gene. The KZR strains that did receive pLAFR1 became Km^S after three repurifications on Tet, as would be expected since the RP1 type plasmids have a low copy number. Once these strains became Km^S, they could be tested for complementation by pRPS404::Tn5.7 plasmids. This conjugation event occurred at high frequency, suggesting that in the construction of pRK290 (Ditta et al., 1980), *sex* was deleted. Table 2 lists the results of the complementation experiments. The *crtC* and *crtD* genes, which when mutated result in green pigmentation, could not be tested for complementation by the pRPS404::Tn5.7 plasmids because the R-prime carries a point mutation in *crtD*. The complementation experiments identified *crtI*, *bchI*, *bchK*, *bchJ*, and *bchL* as distinct genetic loci. Figure 6 is a genetic–physical map of the photosynthesis genes located on the R-prime. The map was constructed from previously published maps of this region (Biel et al., 1983), DNA sequence analysis of regions between 23.0 and 27.8 kb and between 61.5 and 65.5 kb (Youvan et al., 1984), and from mutagenesis and complementation analysis with pRPS404::Tn5.7 plasmids in this study. The *rxcA*, *rxcB*, *rxcC* *lhpA*, *lhpB*, and *bchL* genes, coding for the M, L, and H subunits of the reaction center, the LHI polypeptides, and a 77 amino acid open reading frame associated with bacteriochlorophyll synthesis, respectively, are contained in the sequenced region.

Insertion 28.0 results in a *bchA* phenotype and does not complement *bchC* point mutants but does complement *rxcA* mutations. Mutations in *bchC* result in accumulation of 2-desacetyl-2-hydroxyethyl bacteriochlorophyll *a*, which is an intermediate further along in the bacteriochlorophyll metabolic pathway than accumulated by *bchA* mutants (Biel and Marrs, 1983). Therefore, if *bchA* and *bchC* were on the same operon, a polar mutation upstream from both genes would result in a *bchA* phenotype. Recently, marker-rescue crosses indicated that both *bchA* and *bchC* mutations could be complemented by a subclone of the R-prime containing the Eco RI H fragment (Taylor et al., 1983), which extends from 28.7 to 34.6 kb on the map shown in Figure 6. In that study it was not determined whether the complementation observed for Eco RI subclones was due to transcription initiated from the insert DNA or from the vector DNA. These data are consistent with the interpretation that the promoter for the *bchA* and *bchC* genes is located upstream from Tn5.7 insertion 28.0 and that *bchA* and *bchC* are located on Eco RI fragment H, and that transcription proceeds upstream from insertion 28.0 through *bchA* to *bchC*.

Complementation analysis of *crtE* mutants shows that

Table 2. Complementation Analysis with pRPS404:Tn5.7 Plasmids

pRPS404::Tn5.7 insertions at (kb)	*rxcA*142	*bchC*1007	*crtE*6	*crtE*526	*crtB*4	*crtB*551	*crtA*301	*bchD*1008	*bchD*561	*bchG*527	*bchE*604	*bchF*1003	*bchH*650	*bchK*Ω56.7	*rxcD*Ω65.7	*crtE*Ω34.6	*bchI*Ω41.6	*bchL*Ω61.6
26.3	−	+																
28.0	+	−																
30.3		−																
31.2		−																
31.4		−														+		
33.3		+	+	+												+		
33.4			+	+												+		
33.7			+	+												+		
33.8			−	−												−		
34.6			−	−														
36.4			+	+	+	+										+		
36.8			+	+	+	+										+		
39.8					+	+	+									+		
40.2					+	+	+	+								+		
41.0							−	+									+	
41.6							+	+	+									
43.1							+	−	−	+							−	
43.2							+	−	−	+							−	
44.6								+	+	+								
46.9										+							+	
47.4										+	+							
49.2										+	+						+	
50.8											−	+						
51.0											−	+	+					
51.7											+	+						
51.9											+	+						
53.5A											+	+						
53.5B											+	+		+				
55.0											+	+		+				
55.1											+	−		+				
56.7												+	+					+
58.0												+	+					
58.5											+	+	−	−				
59.7													−	−				
59.8													−	−				
60.0													+	+				
61.6													+	+	+			
63.5															+			+
63.6															−			+
65.6															−			
65.7															−			
67.9															+			

A positive complementation is +, a negative complementation is −, and a space means the experiment was not done.

crtE is not complemented by insertions 33.8 and 34.6. Since both *crtF*Ω33.7 and *crtD*Ω36.4 do complement *crtE* mutants, transcriptional operons including *crtF crtE* and *crtD crtE* can be ruled out. Tn5.7 insertion 39.8 results in a blue-green phenotype and complements two separate point mutations in *crtB,* which also result in a blue-green phenotype. Therefore, the simplest interpretation is that insertion 39.8 identifies a new gene, *crtI.* This gene is not transcribed from *crtA* because polar insertions in *crtA* have the phenotype of *crtA* mutants, which accumulate demethylspheroidene and spheroidene (Scolnik et al., 1980). These compounds are further along in the carotenoid biosynthetic pathway than compounds accumulated by *crtI* mutants. The *crtA* gene appears to be in a transcription unit separate from both *crtI* and *bchI* since it is complemented by insertions *crtI*Ω39.8 and *bchI*Ω41.6 but not by *crtA*Ω41.0.

The *bchI* gene is identified by insertion 41.6. The *bchI*Ω41.6 complements both point mutations in *bchD* yet results in a bacteriochlorophyll$^-$ phenotype. Complementation analysis indicates that *bchD* and *bchI* are in the same operon with transcription in that order (see Table 2). Recently, there was a difference in complementation observed for two *bchD* mutants by the Bam HI H fragment (Taylor et al., 1983), supporting our conclusion that the original *bchD* locus (Yen and Marrs, 1976) actually consists of two genes. The *bchJ* gene is identified by insertion 49.2, is located on the Bam HI I fragment, and is not on

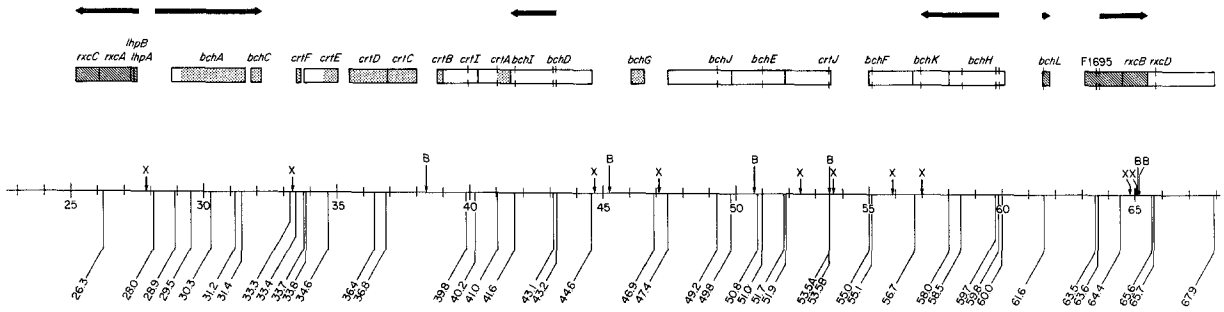

Figure 6. Genetic-Physical Map of the Photosynthetic Gene Cluster Contained on pRPS404

The numbers on the physical map refer to kb moving counterclockwise from the Eco RI site at 0 kb on pRPS404. X and B refer to Xho I and Bgl III restriction sites, which were used to position Tn5.7 insertions. The numbers listed at the ends of vertical lines are the locations of insertions used in this study, +/− 200 bp. The genetic map is shown above. Vertical ticks refer to insertions, either in the locus indicated or upstream. The boundaries for the loci, indicated by full vertical lines, were determined by DNA sequencing (Youvan et al., 1984), dark shading; previously published loci determined from gene-transfer agent crosses (Yen and Marrs, 1976), light shading; or Tn5.7 mutagenesis and complementation analysis, no shading. The arrows above postulate direction and extent of transcription for some photosynthesis genes.

an operon with any of the *bch* genes tested. This insertion complements point mutations in *bchG* and *bchE*. The *bchJ*Ω49.2 strain excretes a porphyrin precursor into the growth medium unlike *bchE* mutants.

Insertions 53.5A and 53.5B appear to be insertions at the same site, as judged by agarose gel electrophoresis, which has about a 50 bp resolution in the <2 kb range (Figure 7). However, the two insertions are in the opposite orientation. Insertion 53.5A results in a blue-green phenotype and 53.5B is bacteriochlorophyll$^-$. It is unlikely that the orientation of insertion is the cause of the two phenotypes because the ends of Tn5.7 are identical for 1500 bp at the ends. Probably the two insertions differ by less than 50 bp and are in two distinct genes. The bacteriochlorophyll$^-$ phenotype of the 53.5B insertion mutant could not be complemented by any of the pRPS404::Tn5.7 plasmids tested, even after it had been shown to have recombined and deleted the wild-type Eco RI fragment. Therefore, it is omitted from the genetic map in Figure 6 as a *bch* locus.

Insertion 56.7 results in a bacteriochlorophyll$^-$ phenotype yet complements both *bchH* and *bchF* mutants and is designated *bchK*. Insertion *bchF*Ω55.1 is the only pRPS404::Tn5.7 plasmid that did not complement point mutations in *bchF* and so *bchF* is not in the same operon as *bchK* and *bchH*. The *bchK*Ω56.7 mutant is not complemented by insertions in *bchH*, but *bchH* point mutants are complemented by *bchK*Ω56.7. Therefore it is postulated that the two genes are transcribed together in the order *bchH bchK*, shown in Figure 6.

In the region to the left of *rxcD* in Figure 6 there is a division in phenotypes and complementation groups around 63.5 kb. Inspection of this region for consensus promoter sequences from enteric bacteria (Hawley and McClure, 1983) shows no significant homologies. It has not been determined what an oxygen-regulated promoter consists of in R. capsulata. A comparison of the 5′ DNA sequence for the M subunit gene from R. spheroides (Williams et al., 1983); R. capsulata *lhpA*, F1696, and the region around 63.5 kb revealed the sequence homologies shown in Figure 8. The function of this conserved region

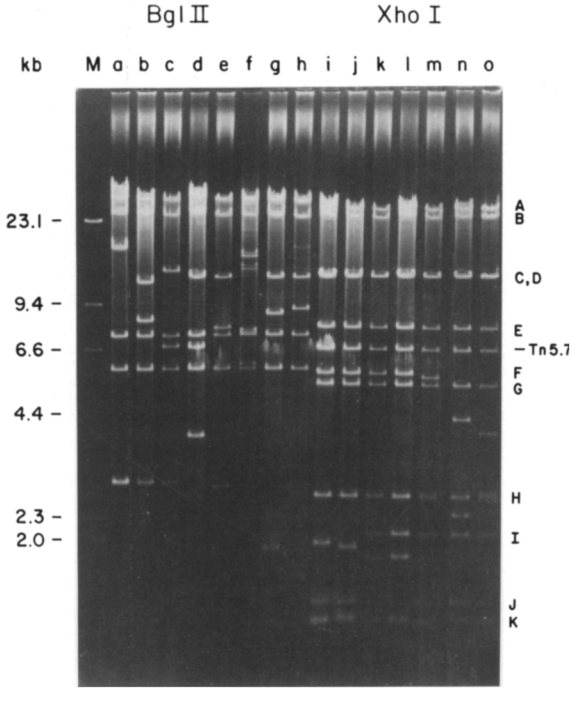

Figure 7. Agarose Gel Electrophoresis of pRPS404::Tn5.7 Plasmids

Plasmids were purified from E. coli strains according to Experimental Procedures, digested with restriction enzymes Bgl II (lanes a–h) or Xho I (lanes i–o), and electrophoresed in a 0.7% agarose gel so that the order of Tn5.7 insertions between 50.8 kb and 55.1 kb on pRPS404 could be determined to +/−50 bp. Letters A–K on the right refer to fragments of the plasmid after digestion with Xho I, the 6.6 kb internal Tn5.7 band is indicated. Lane M refers to molecular weight markers, indicated on the left. Plasmids shown with Tn5.7 insertions are lanes a and i, 55.1; b and j, 55.0; c and k, 53.5B; d and l, 53.5A; e and m; 51.7; f, 51.9; g and n, 51.0; h and o, 50.8.

is not known but it might be involved in some aspect of promoter recognition. One model consistent with the data is that the reduced reaction center incorporation by F1696Ω63.6 and F1696Ω64.4 is due to polar inactivation of the *rxcB* gene and that this gene has two promoters, one that is between insertions 63.5 and 63.6 and another

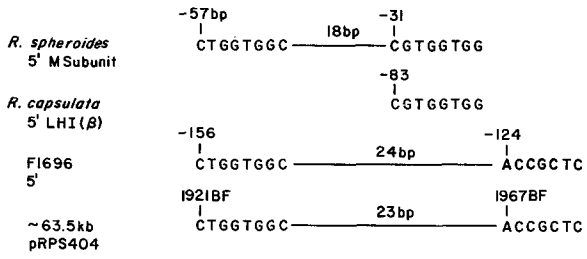

Figure 8. Comparison of DNA Sequences between R. spheroides and R. capsulata

Regions located 5′ to the R. spheroides M subunit of the RC gene are compared with R. capsulata 5′ region to lhpA, 5′ to the F1696 open reading frame, and the region around 63.5 kb (+/−200 bp) in the Bam HI F fragment, where insertions of Tn5.7 indicate the beginning of a transcriptional unit. The A of the ATG codon starting translation is numbered +1. Regions upstream have negative numbers. The region around 63.5 kb is identified as 1921 bp from the Bam HI site at around 61.6 kb on pRPS404.

just before *rxcB*. Preliminary experiments in our laboratory (P. O'Brien) indicate that there is a decreased amount of RNA made that is specific to the *rxcB* gene in the F1696Ω63.6 but not in the F1696Ω63.5 R. capsulata strains.

Transposon Tn5.7 mutagenesis of a photosynthetic gene cluster from R. capsulata has provided a number of mutations that may aid in the understanding of both bacterial and higher plant photosynthesis. The target DNA sequence may be subcloned by direct selection for the Tn5.7 antibiotic-resistance markers. Recently found homologies between R. capsulata and the 32 kd thylakoid membrane protein (Hearst and Sauer, 1984) suggest that through heterologous hybridizations, higher plant photosynthesis genes may be cloned using bacterial sequences. Mutations in the bacteriochlorophyll biosynthetic genes provide a number of intermediates that may help in elucidating the biosynthetic pathway for bacteriochlorophyll and chlorophyll biosynthesis. A regulatory mutation affecting reaction centers, as well as a 44 kd heme-staining polypeptide, has been identified and may provide clues to the regulatory interactions necessary for the differentiation of photosynthetic membranes. Complementation analysis has led to the postulation of transcriptional units containing reaction-center structural genes and bacteriochlorophyll and carotenoid biosynthetic genes.

Experimental Procedures

Media and Cultivation

E. coli strains were grown on L medium. R. capsulata strains were grown on plates chemeheterotrophically on PYE medium or minimal RCV medium (Yen and Marrs, 1977), or in liquid in RCV supplemented with 50 mM dimethyl sulfoxide, 0.6% (w/v) dextrose, and 0.5% (w/v) sodium pyruvate (Yen and Marrs, 1977). Photosynthetic growth-defective (PS^-) mutants were screened by streaking replica PYE plates. One plate was incubated aerobically in the dark (permissive for [PS^-] and the duplicate plate was incubated under photosynthetic conditions, with both PS+/− controls, in an anaerobic jar (BBL GasPak) within a temperature-controlled light-box (3 mW/cm^2). Antibiotics were used at the following concentrations (μg/ml): kanamycin 15, streptomycin 10, spectinomycin 10, tetracycline 15.

Conjugal Matings

pRPS404::Tn5.7 plasmids were conjugated in spots on PYE agar plates between E. coli donors and R. capsulata recipients at 32°C. A 1:10 ratio of donor:recipient was used, the donors being in mid-log phase. The spots were incubated overnight. For the KZR library construction, the transconjugants were selected on RCV containing Sm (5 day incubations). After two repurifications on this media, repurification continued on PYE containing Sp (3 day incubations). Most KZR strains went through about ten repurifications on this media to ensure that phenotypic analysis was not performed on merodiploids. For complementation analysis of R. capsulata point mutants and transposon-generated mutants by pRPS404::Tn5.7 plasmids, transconjugant selection was on RCV containing 10 μg/ml Kanamycin. For conjugation of pLAFR1 into KZR strains, triparental matings were performed with pRK2013, pLAFR1, and KZR recipients as described above for pRPS404 transfers. Selection of transconjugants was performed on RCV containing Tet. The efficiency of transfer was variable from 10^{-5}–0. After two repurifications on RCV 10 μg/ml tetracycline, and an additional repurification on PYE Tet, all strains that received pLAFR1 became Km^S.

Complementation Analysis

R. capsulata mutants were tested for complementation by various pRPS404::Tn5.7 plasmids by performing conjugations as described above. Complementation of blue-green mutants was tested simply by observation of colony pigmentation of transconjugants. For $Bchl^-$ mutants, a colony from each merodiploid strain was resuspended in liquid RCV media and serially diluted to 10^{-7}, then spotted on replica RCV plates containing 10 μg/ml Km. One set was incubated aerobically in the dark and one set was grown photosynthetically. A positive complementation resulted in a difference of 10^1 to 10^2 colonies between the PS^+ plate and the PS^- plate. A negative complementation resulted in a difference between 10^4 to 10^6 colonies between the two plates. The *bchA* mutations were difficult to complement by any pRPS404::Tn5.7 plasmids, i.e., there was no clear designation between positive or negative complementation and therefore they are not included in the results.

Nucleic Acid Isolation and Analysis

Plasmid DNA was isolated essentially as described (Birnboim and Doly, 1979). Total DNA was isolated as described (Marmur, 1961) but with an isopropanol precipitation included. DNA samples were digested with restriction enzymes according to BRL. Agarose gels, 0.7%, transfer of DNA to nitrocellulose, nick-translation of pRPS404, and hybridization was performed as described (Maniatis et al., 1975).

Absorption Spectroscopy and SDS-PAGE

Chromatophores were isolated as described (Youvan et al., 1983). Absorption spectroscopy was performed on a cleared cell lysate on a Varian 2300 spectrophotometer. Bacteriochlorophyll precursor absorption maxima were determined in methanol extracts of whole-cell pellets 1 ml CH_3OH/g cells. SDS-PAGE was performed on twice-washed chromatophores as described (Youvan et al., 1983). Staining for heme-containing polypeptides was performed as described (Hoyer-Hansen, 1980).

DNA Homology Searches

Searches for DNA homology between R. capsulata and R. spheroides were done using a high-speed homology matrix program (Pustell and Kafatos, 1982).

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