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Alignment of Genetic and Restriction Maps of the Photosynthesis Region of the *Rhodopseudomonas capsulata* Chromosome by a Conjugation-Mediated Marker Rescue Technique

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The restriction map of a 46-kilobase fragment of the *Rhodopseudomonas capsulata* chromosome was aligned with the genetic map of the photosynthesis region of that chromosome by a marker rescue technique. Marker rescue was effected by mobilization of vectors bearing fragments of *R. capsulata* DNA from *Escherichia coli* to a set of *R. capsulata* mutants. Plasmids pDPT51 and pDPT55 were constructed to mediate the intergeneric mobilization of pBR322 derivatives, and a mutant of *R. capsulata* with improved intergeneric recipient activity was isolated. Four previously unmapped genes affecting bacteriochlorophyll synthesis and two genes affecting photochemical reaction center synthesis have been located by marker rescue. Some of the fragments of *R. capsulata* DNA are capable of vector-independent complementation, implying that promoters are located on these fragments. Other fragments complement only in one orientation of insertion in the vector, implying transcription from promotors on the vectors and thereby fixing the direction of transcription for those fragments. Still other fragments of DNA show rescue only via recombination between homologous plasmid-borne DNA fragments and chromosomal mutations. The physical dimensions of the genetic map are 3.0 megadaltons per map unit, which agrees with previous estimates based on the size of the *R. capsulata* gene transfer agent.

Synthesis of the photosynthetic apparatus of *Rhodopseudomonas capsulata* is regulated by oxygen tension. At low pO_2, three distinct types of pigment-protein complexes are synthesized and integrated into the cytoplasmic membrane, bringing about its differentiation into intracytoplasmic photosynthetic structures. The synthesis of photosynthetic membranes is inhibited by elevated pO_2 (7). A genetic map of many mutations affecting the photosynthetic apparatus was constructed, and relatively tight clustering of the photosynthetic pigment genes was demonstrated (15, 24). It has recently become possible to analyze the physical arrangement of those and neighboring genes by virtue of the isolation of R-factor derivatives that have integrated a portion of the *R. capsulata* chromosome including the genes for photosynthesis. These R-prime factors are capable of complementing virtually all known mutations affecting the differentiation of photosynthetic from cytoplasmic membranes (11).

In this paper we describe the cloning of restriction fragments of one R-prime plasmid, pRPS404, in vectors derived by adding a kanamycin resistance gene to pBR322. The kanamycin marker is well expressed in *R. capsulata*, whereas the ampicillin and tetracycline markers of pBR322 are not. The return of cloned fragments to *R. capsulata* for marker rescue analysis was achieved by mobilization from *Escherichia coli* mediated by a plasmid constructed by fusion of a promiscuous plasmid with a ColE1 derivative. The intergeneric mobilization was facilitated by isolation of a mutant strain of *R. capsulata* that is more efficient than the wild type as a recipient of DNA replicated in *E. coli*, but unchanged as a recipient for DNA from *R. capsulata*. By testing the set of cloned pRPS404 restriction fragments against a set of *R. capsulata* mutants, an unambiguous alignment of the restriction and genetic maps was achieved. The results show that one map unit on the gene

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transfer agent-based genetic map is equivalent to 3.0 megadaltons. This finding is in excellent accord with the prediction that one map unit would represent the amount of DNA contained in the gene transfer agent particle (3 megadaltons) (23). Hitherto unmapped loci, involved in bacteriochlorophyll synthesis and reaction center formation, have been localized by marker rescue. In addition, the direction of transcription of part of the region and the approximate locations of several promoters have been established by analyzing the orientation of recombinant DNA molecules that show complementation in marker rescue experiments.

MATERIALS AND METHODS

Bacterial strains, plasmids, and culture conditions. The bacterial strains and plasmids utilized in this study are listed in Table 1. Peptone-yeast extract and malate-minimal (RCV) media were used to support growth of *R. capsulata*, and *E. coli* strains were grown on LC broth as previously described (11). Minimal agar plates supplemented with 0.5% Casamino Acids (Difco Laboratories, Detroit, Mich.) for growth of *E. coli* were prepared as described by Miller (13). Ampicillin and tetracycline were used at 25 μg/ml and kanamycin was used at 50 μg/ml for selection of resistant *E. coli*. Kanamycin was used at 10 μg/ml to select resistant *R. capsulata*.

Isolation and manipulation of plasmid DNA. Plasmids were isolated and recombinant plasmids constructed by previously described technique (20). Restriction mapping of fragments of pRPS404 is described elsewhere (W. G. Clark, D. P. Taylor, J. M. Shively, S. N. Cohen, and B. L. Marrs, submitted for publication).

*Bam*HI restriction fragments of pRPS404 were cloned by ligating into pDPT42 that had been cut with *Bam*HI. Alkaline phosphatase was used in some experiments to minimize reclosing of plasmids without inserts (21). Ampicillin- and kanamycin-resistant transformants of *E. coli* C600 were then screened for Tc^s, and the resultant clones examined for the *Bam*HI digestion patterns of plasmids they bore. Representatives carrying most of the *Bam*HI fragments of pRPS404 were found. One fragment, *Bam*HI-C, was not found and attempts to clone it directly were not successful, so the following procedure was used to clone that portion of *Bam*HI-C that was not covered by *Eco*RI-Q and *Eco*RI-H. An *Eco*RI-*Bam*HI double digest of pRPS404 was ligated with an *Eco*RI-*Bam*HI-*Hin*dIII triple digest of pBR322. The *Hin*dIII digestion minimizes the probability of reassembling the starting plasmid. The ligation mixture was used to transform *E. coli* HB101, and Ap^r transformants were screened for Tc^s. Plasmid DNA was prepared from those clones, and the *EcoRI-Bam*HI digestion patterns were compared with that of pRPS404. The desired *Eco*RI-BamHI fragment was located by the procedure of Southern (17) by probing with nick-translation-labeled *Bam*HI-C cut from low-melting-point agarose gels. A plasmid, pRPSEB1, bearing the appropriate inserted fragment was identified and digested with *Eco*RI and ligated to an *Eco*RI-*Bam*HI double digest of pDPT42. The mixture was used to transform *E. coli* HB101, and Km^r transformants were selected. One such clone contained plasmid pRPSEB2 in which the 7-kilobase (kb) Km^r-determining *Eco*RI fragment from pDPT42 has been inserted at the *Eco*RI site of pRPSEB1. The orientation of this fragment in pRPSEB2 is reversed compared with that in pDPT42.

Marker rescue crosses. Vector plasmids pDPT42 or pDPT44 or their derivatives bearing inserts of DNA from pRPS404 were transformed into *E. coli* strains HB101, C600, or W3110 as previously described (5). Mobilizing plasmids (pDPT51 or pDPT55) were then introduced by conjugation into each *E. coli* strain bearing the vector or recombinant derivative. Twenty microliters of a mixture of donor (10^5 to 10^7 CFU/ml) and recipient (10^9 CFU/ml) cells were spotted on cured minimal agar plates supplemented with Casamino Acids. After allowing 2 h at 35°C for mating and phenotypic expression, plates were spread with 0.1 ml of G-buffer (16) and overlayered with soft agar containing antibiotics to bring the final concentrations to 50 μg of trimethoprim per ml and 25 μg of kanamycin sulfate per ml to select for the presence of both the mobilizing plasmid and the vector. Exconjugants were isolated and used as donors in intergeneric marker rescue crosses with *R. capsulata* mutants as follows. Two 50-μl spots of a mixture of about 10^9 CFU of each donor and recipient per ml were dried on the surface of cured RCV plates and then incubated for 4 h at 35°C, spread with 0.1 ml of G-buffer, and overlayered with kanamycin sulfate-containing soft agar to a final concentration of 10 μg/ml. Plates were then incubated aerobically in darkness at 35°C for 4 to 5 days.

Gene transfer agent-mediated crosses. Gene transfer agent-mediated crosses were performed as previously described (24). A cell-free filtrate of the donor culture was incubated with recipient cells, and after time was allowed for gene transfer agent uptake, cells were plated and subsequently challenged for acquisition of recombinant phenotypes.

RESULTS

Cloning R-prime plasmid restriction fragments. *Bam*HI and *Eco*RI restriction endonuclease digests of the 112-kb R-prime pRPS404 gave rise to 15 and 18 fragments, respectively (Clark et al., submitted for publication). These fragments were cloned in plasmid vectors by standard techniques. Since our experimental plan entailed eventually returning the cloned fragments to *R. capsulata*, it was necessary to construct vectors with a marker that could be expressed in that host. pDPT42 and pDPT44 were constructed in vitro by introducing the kanamycin resistance determinant of plasmid pDPT31 (20) into pBR322 (Fig. 1). The tetracycline resistance marker of pBR322 does not function well in *R. capsulata*, and the ampicillin resistance marker does not function at all (unpublished observations). pDPT42 has the 7.0-kb *Eco*RI Km^r fragment from pDPT31 spliced into the *Eco*RI site of pBR322, creating a vehicle suitable for cloning *Bam*HI fragments. pDPT44 has the 5.1-kb *Eco*RI-*Sal*I fragment from

TABLE 1. Bacterial strains and plasmids

Designation	Relevant markers and properties	Comments and references[a]
E. coli		
HB101	*recA pro leu thr lacY* Str^r Res^-(K), Mod^-(K)	(3)
C600	*thr-1 leu-6 thi-1 supE44 lacY1 tonA21* (λ^-) (F^-) Res^-(K)	(20)
1287.1	Str^r, prototroph	From D. Berg
W3110	Prototroph	From H. Lozeron
R. capsulata		
BP503	*crtF129 hsd-1 str-2*	Demethylspheroidene accumulated, derived from BY16512 by GTA
BPY27	*bchG527 crtF129*	P760 accumulated, Psg^- (11)
BPY38	*bchA538 crtF129*	P670 accumulated, Psg^-, derived from PY1291 (15)
BPY61	*bchD561 crtF129*	Trace amounts of normal BChl present, Psg^-, derived from PY1291 (15)
BPY72	*bchF572 crtF129*	P730 accumulated, Psg^-, derived from PY1291 (15)
BPY91	*bchC591 crtF129*	P710 accumulated, Psg^- (11)
BPY381	*bchA538 crtF129 rif-10*	Rif^r derivative of BPY38 by GTA
BPY612	*bchD561 crtF129 rif-10*	Rif^r derivative of BPY61 by GTA
BRP3	*bchA603 crtF129 hsd-1 str-2*	P670 accumulated, Psg^-, derived from BP503
BRP4	*bchE604 crtF129 hsd-1 str-2*	P590 accumulated, Psg^-, derived from BP503
BRP15	*bchH615 crtF129 hsd-1 str-2*	No BChl or precursor, Psg^-, derived from BP503
BRP33	*bchG633 crtF129 hsd-1 str-2*	P760 accumulated, Psg^-, derived from BP503
BRP46	*bchG646 crtF129 hsd-1 str-2*	P760 accumulated, Psg^-, derived from BP503
BRP50	*bchH650 crtF129 hsd-1 str-2*	No BChl or precursor, Psg^-, derived from BP503
BRP54	*bchE654 crtF129 hsd-1 str-2*	P590 accumulated, Psg^-, derived from BP503
BY1651	*bchA165 crtB4 str-2*	P670 accumulated, no carotenoids, Psg^- (11)
BY16510	*bchA165 crtB4 hsd-1 str-2*	This work, derived from BY1651
BY16511	*crtB4 hsd-1 str-2*	Psg^+, derived from BY16510 by GTA
BY16512	*crtB4 crtF129 hsd-1 str-2*	No carotenoids, Psg^+, derived from BY16510 by GTA from PY1291 (15)
M124	*rxcB124 aer103r124*	Psg^-, reaction center deficient, derived from M50 (25)
MB1003	*bchF1003*	P730 accumulated, Psg^-
MB1007	*bchC1007*	P710 accumulated, Psg^-
MB1008	*bchD1008*	Trace amounts of normal BChl, Psg^- (11)
PAS100	*hsd-1 str-2*	Psg^+, wild-type carotenoids, derived from BY16511 by GTA
PAS108	*rxcA108 hsd-1 str-2*	Psg^-, derived from PAS100 by tetracycline suicide
SB1003	*rif-10*	Rif^r, wild-type photopigments (11)
Y5	*rxcA5 aer103r5*	Psg^-, reaction center deficient, derived from M50 (25)
Y9	*rxcA9 aer103r9*	Psg^-, reaction center deficient, derived from M50 (25)
Y142	*rxcA142 str-2*	Psg^-, reaction center deficient (11)
Y402	*rif-10*	Rif^r, GTA overproducer (23)
Plasmid		
ColE1::Tn*5*	Km^r	Obtained from D. Berg
pBLM2	Km^r Cma^+	Derived from RP1 (11)
pDPT31	Km^r Tc^r	Derived from pSC301 (20)
pDPT42	Km^r Tc^r Ap^r	This work, a vector
pDPT44	Km^r Ap^r	This work, a vector
pDPT51	Tp^r Ap^r	This work, a mobilizing plasmid
pDPT55	Tp^r Ap^r	This work, a mobilizing plasmid
pRPS404	Km^r Psg	R-prime bearing genes for photosynthesis (11)
pRPSB4	Km^r Tc^s Ap^r	*Bam*HI-H cloned in pDPT42
pRPSB5	Km^r Tc^s Ap^r	*Bam*HI-D cloned in pDPT42
pRPSB9	Km^r Tc^s Ap^r	*Bam*HI-I cloned in pDPT42
pRPSB10	Km^r Tc^s Ap^r	*Bam*HI-G and *Bam*HI-F cloned in pDPT42

TABLE 1—*Continued*

Designation	Relevant markers and properties	Comments and references[a]
pRPSB31	Km^r Tc^s Ap^r	*Bam*HI-G and *Bam*HI-E cloned in pDPT42
pRPSB104	Km^r Tc^s Ap^r	*Bam*HI-F cloned in pDPT42
pRPSB105	Km^r Tc^s Ap^r	*Bam*HI-G cloned in pDPT42
pRPSB108	Km^r Tc^s Ap^r	*Bam*HI-G cloned in pDPT42
pRPSB40	Km^r Tc^s Ap^r	*Bam*HI-J and *Bam*HI-F cloned in pDPT42
pRPSE30	Km^r Ap^r	*Eco*RI-H cloned in pDPT44
pRPSE2	Km^r Ap^r	*Eco*RI-F cloned in pDPT44
pRPSEB1	Ap^r Tc^s	*Bam*HI-C–*Eco*RI-A cloned in pBR322
pRPSEB2	Km^r Ap^r Tc^s	Km^r, from pDPT42 cloned in pRPSEB1
R751	Tp^r	(12), promiscuous R-factor
RSF2011	Ap^r	ColE1::Tn*3* (14)
RSF2233	Ap^r	ColE1::Tn*3* (14)

[a] Abbreviations: P590, P630, P670, P710, P730, and P760 refer to pigments believed to be percursors of bacteriochlorophyll (BChl); "by GTA" means constructed via gene transfer agent-mediated genetic exchange; Psg^-, incapable of photosynthetic growth; Rif^r, rifampicin resistant; Km^r, kanamycin resistant; Tc^r, tetracycline resistant; Ap^r, ampicillin resistant; *Bam*HI-H, *Bam*HI-D, *Eco*RI-H, *Eco*RI-F, etc., restriction fragments of pRPS404; *Bam*HI-C–*Eco*RI-A, the *Eco*RI-*Bam*HI restriction fragment common to both *Bam*HI-C and *Eco*RI-A; Cma^+, capable of chromosome mobilization.

pDPT31 replacing the 650-base-pair *Eco*RI-*Sal*I piece of pBR322, creating a vehicle suitable for cloning *Eco*RI fragments.

A *Bam*HI digestion of pRPS404 was ligated into *Bam*HI-cut pDPT42 and used to transform *E. coli* strain C600 (r^-, m^+). The resulting ampicillin- and kanamycin-resistant, tetracycline-sensitive colonies were screened for plasmids bearing *Bam*HI inserts in pDPT42 by isolation, *Bam*HI digestion, and gel electrophoresis. *Eco*RI fragments of pRPS404 were cloned into pDPT44 which had been cut with *Eco*RI and treated with alkaline phosphatase to improve recovery of recombinant clones (21). In this way collections of *E. coli* strains carrying most of the *Bam*HI and *Eco*RI fragments of pRPS404 were established.

Construction of a mobilizing plasmid. Our strategy to identify the genetic content of the cloned fragments was to mobilize the recombinant plasmids into *R. capsulata* mutants and to test for complementation or recombination. To achieve this it was necessary to create a mobilizing plasmid that could be transferred into each *E. coli* strain containing the cloned *R. capsulata* fragments and could then mediate the intergeneric transfer of the recombinant molecules into *R. capsulata*. R751 was chosen as the promiscuous mobilizing plasmid because it belongs to the P-1 incompatibility group, it lacks Km^r, and it bears a trimethoprim resistance (Tp^r) marker. The Tp^r marker allows the selection of exconjugants that have received the mobilizing plasmid during the construction of *E. coli* donor strains.

pBR322 and its derivatives pDPT42 and pDTP44 cannot be mobilized by R751 or related plasmids unless certain gene products for mobilization are supplied from another source. The gene products needed to mobilize pBR322 can be supplied in trans by ColE1 or derivatives of ColE1 that bear Tn*3* inserted at sites outside the mobilization genes, such as RSF2011 and RSF2233 (22). There is unidirectional incompatibility between pBR322 and ColE1, with the latter almost always being lost from *E. coli* initially bearing both plasmids (9). To circumvent this potential problem and to facilitate strain construction, plasmids pDPT51 and pDPT55 were constructed by fusing R751 to RSF2011 and RSF2233, respectively. Fusion was accomplished by ligating *Bam*HI digests of either RSF2011 or RSF2223 with a *Bgl*II digest of R751. The *Bam*HI site in RSF2233 and RSF2011 is in the *tnpR* gene of Tn3 (10) and thus does not affect mobilization, and the *Bgl*II site in R751 is in a region not required for either replication or conjugal transfer of the plasmid (12). Both pDPT51 and pDPT55 functioned to mobilize pDPT42 from C600(pDPT42) to other strains of *E. coli* at moderate frequencies (10^{-3} to 10^{-2} transfers per donor), but much lower rates of transfer were observed when *R. capsulata* strains were used as recipients. Furthermore, the resulting Km^r *R. capsulata* clones were unstable, rapidly losing Km^r when grown in media without kanamycin.

Isolation of an *R. capsulata* strain with enhanced recipient activity. Five Km^r clones of *R. capsulata* were isolated from the mating of C600 (pDPT55 + pRPSE18) × BY1651, and Km^s segregants from each clone were picked after a short period of growth. pBR322 derivatives were much less stable than ColE1 in *R. capsulata*, and they gave rise to spontaneous segregants at very high frequencies. One Km^s segregant, BY16510, when tested as a recipient for Km^r in a

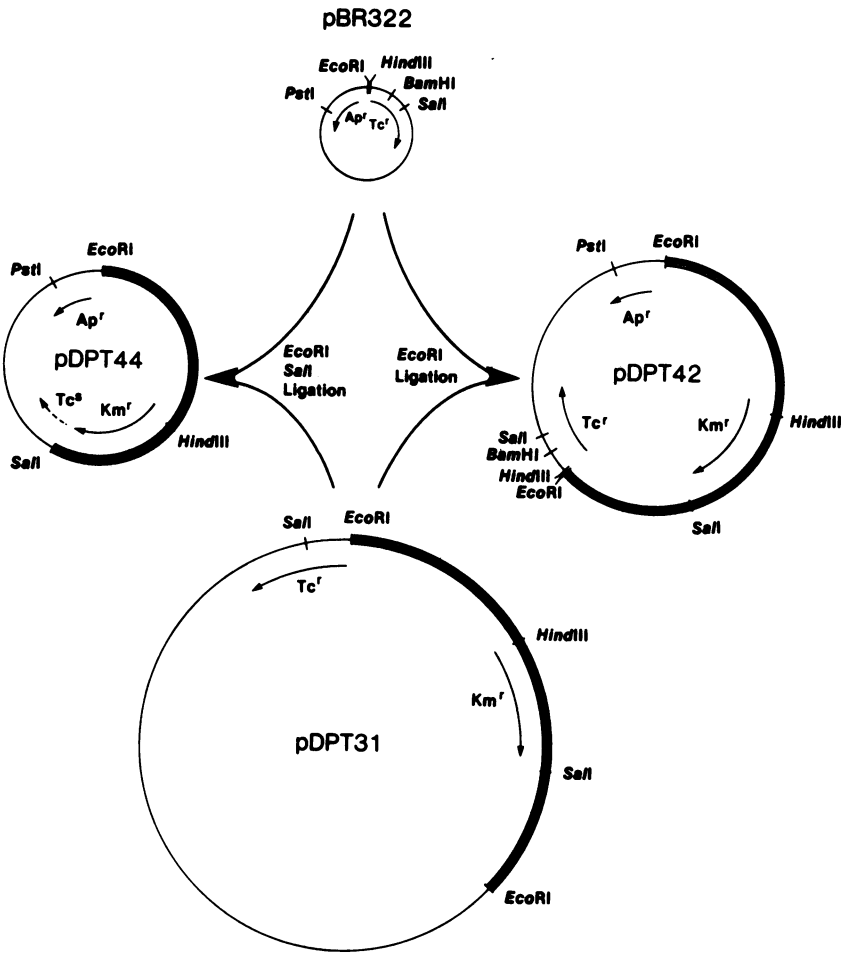

FIG. 1. Construction of the vectors pDPT42 and pDPT44. The 11.4-kb plasmid pDPT42 was constructed by ligating *Eco*RI restriction endonuclease digests of pBR322 and pDPT31. Km^r and Ap^r C600 transformants were selected and screened for Tc^r. The orientation of the fragment bearing the Km^r determinant (shaded portion) was determined by digestions with *Sal*I and *Hin*dIII. The 8.8-kb plasmid pDPT44 was constructed by ligating the *Eco*RI-*Sal*I double digests of pBR322 and pDPT31. Km^r and Ap^r transformants of C600 were selected and screened for Tc^s. The positions and directions of transcription of the antibiotic resistance markers are indicated by arrows. Restriction sites for other enzymes in the Km^r-bearing fragment are described in references 1, 2, and 8.

second round of mating with the same type donor, showed 30 to 1,000 times more Km^r exconjugants per donor compared to BY1651. To test the basis of this enhanced recipient activity, BY1651 and By16510 were used as recipients in parallel genetic transfer experiments (Table 2). In matings in which DNA was mobilized from *E. coli* to *R. capsulata*, BY16510 was a better recipient than BY1651, but the two were equal in recipient ability when the donor strain was *R. capsulata*. This suggests that a restriction system has been inactivated in BY16510, and the mutation conferring this phenotype was therefore named *hsd-1*. Strains bearing this mutation were used as recipients wherever possible.

Construction of a collection of *R. capsulata* mutants with enhanced recipient activity. To facilitate the screening of restriction fragments for marker rescue activity, we generated a collection of pigment mutants, each carrying the mutation for enhanced recipient activity. Since there was no easy way to transfer the *hsd-1* mutation into previously described strains, we chose to generate new pigment mutations in a strain with enhanced recipient activity. This was accomplished by transferring the wild-type alleles of the *crtB* and *bchA* lesions into BY16510 and

TABLE 2. Recipient activity of strains BY1651 and BY16510

Donors[a]	Recipient activity[b]	
	BY1651	BY16510
E. coli		
C600(pDPT51 + pRPSB105)	5.0×10^{-8}	1.2×10^{-6}
1287.1(pDPT55 + ColE1::Tn5)	2.0×10^{-7}	3.0×10^{-4}
HB101(pRPS404)	1.3×10^{-4}	1.7×10^{-2}
HB101(pBLM2)	1.0×10^{-4}	3.6×10^{-3}
R. capsulata		
SB1003(pBLM2)	2.1×10^{-2}	1.6×10^{-2}
Y402, gene transfer agent	1.8×10^{2}	2.5×10^{2}

[a] Each donor was used in a conjugal cross with an excess of the indicated recipient, except strain Y402, which served as a donor of gene transfer agent which was titered with excess recipient.

[b] For conjugal crosses, activity is kanamycin-resistant recombinants per donor cell. For the gene transfer agent cross, activity is the number of Rif^r recombinants induced by 1.7×10^{-2} μl of a cell-free filtrate of the donor culture.

introducing a lesion in *crtF*. Strains bearing *crtF* mutations are rich sources of additional mutations affecting pigment synthesis, because of the following properties. CrtF mutants lack the ability to methylate the tertiary hydroxyl group of the carotenoids hydroxyneurosporene (1,2,7′,8′-tetrahydro-Ψ-Ψ-caroten-1-ol), demethyspheroidene (3,4-didehydro-1,2,7′,8′-tetrahydro-Ψ-Ψ-caroten-1-ol), and demethylspheroidenone (1-hydroxy-3,4-didehydro-1,2,7′,8′-tetrohydro-Ψ-Ψ-caroten-2-one) (15). The last two compounds are accumulated in CrtF mutants, and they appear to be deleterious to *R. capsulata* under some growth conditions. Under those conditions, mutants that no longer accumulate the demethylated carotenoids quickly overgrow the population. Mutation rates to antibiotic resistance are not enhanced in CrtF mutants; therefore, we feel that the phenomenon is one of selection rather than mutagenesis. Secondary mutations that confer a selective advantage to CrtF mutants include blocks in all earlier steps of the carotenoid biosynthetic pathway and all steps in the bacteriochlorophyll biosynthetic pathway. Blocks in bacteriochlorophyll synthesis result in lowered carotenoid levels, presumably as a response to the virtual absence of reaction center and antennae pigment-binding proteins in cells without bacteriochlorophyll (19). When CrtF mutants are plated in minimal medium, each colony develops a particolored appearance upon prolonged incubation (1 week). Each colony that is picked and respread gives rise to a variety of different mutant types, each bearing the original *crtF* mutation plus a second mutation. These double mutants are stable, and the new pigment mutations are characterized by standard techniques. The collection of mutants generated in this way facilitated marker rescue experiments.

Marker rescue experiments. Recombinant plasmids bearing various restriction fragments of pRPS404 were mobilized from *E. coli* C600 derivatives, which also contained pDPT51, into a variety of *R. capsulata* mutants. Recombinant plasmid- bearing *R. capsulata* exconjugants were isolated by selection for Km^r and prototrophy. Colonies of plasmid-bearing *R. capsulata* mutants were examined for pigment phenotype to detect genetic complementation, and samples were suspended and plated to detect recombination-based marker rescue. The results of these experiments are summarized in Table 3. Recombination was detected among restriction fragments and mutations blocking photosynthetic ability by plating suspended plasmid-bearing *R. capsulata* cells in photosynthetic growth conditions without kanamycin. The frequency of recombinants per cell was generally about 10^{-2}. When complementation occurred, most of the cells in each Km^r colony were capable of photosynthetic growth, although pigment production was not always restored to wild-type levels.

Complementation implies in situ transcription of the plasmid-borne fragments. One example of complementation was observed when pRPSB105, bearing the *Bam*HI-G fragment, was mobilized into strain BY16511, which lacks carotenoid production due to the *crtB4* mutation. To test whether transcription of the *Bam*HI-G fragment was initiated at a promotor on the insert DNA or at a promotor on the vector, plasmids with the *Bam*HI-G insert in each of the two possible orientations were constructed. One of these, pRPSB105, showed complementation, whereas the other, pRPSB108, did not. pRPSB108 gave rise to recombinants in strain BY16511 that regained wild-type carotenoid production. It may thus be concluded that a promotor on the vector is responsible for transcription of the *crtB* gene of plasmid pRPSB105. It is known that the *Bam*HI restriction site of pBR322, which is the site of insertion of pRPS404 fragments in pDPT42, is downstream about 330 base pairs from the Tc^r promoter (14). A further suggestion that the Tc^r promoter is the site of initiation of transcription comes from the observation that when the 4.0-kb BamF fragment is inserted on the side of the *Bam*HI-G fragment opposite from the Tc^r promoter, as in pRPSB10, complementation ability is not lost. If a promoter of the vector on the opposite side of *Bam*HI-G from the Tc^r promoter were in fact responsible for *crtB* gene expression, this large

TABLE 3. Marker rescue crosses[a]

Mutations	pRPSEB2 (EB2[b])	pRPSE30 (*Eco*RI-H)	pRPSB40 (*Bam*HI-F, *Bam*HI-J)	pRPSB105 (*Bam*HI-G)	pRPSB4 (*Bam*HI-H)	pRPSB31 (*Bam*HI-G, *Bam*HI-E)	pRPSB9 (*Bam*HI-I)	pRPSB5 (*Bam*HI-D)	pRPSB10 (*Bam*HI-G, *Bam*HI-F)	pRPSE2 (*Eco*RI-F)
rxcA108	+	−								
rxcA142	C	−		−		−	−	−	−	
bchA603		C	−	−		−			−	
bchA165		C			−		−	−		
bchC591		C	−	−					−	
bchC1007		C	+		−					
crtB4				C	−	+	−	−	C[c]	
bchD1008					C	−				
bchD561				−	−	−			−	
bchG646						+				
bchG633					−	+		−		
bchE654							C			
bchE604				−	−	−	C	−		
bchH615								+		
bchH650				−	−	−		+		
bchF1003						−		C		
bchF572						−		C		
rxcB124	−									C

[a] Symbols: +, recombination; −, no recombination or complementation; C, complementation; no entry indicates not tested.

[b] This restriction fragment is the piece of DNA in common between *Eco*RI-B and *Bam*HI-C.

[c] The *Bam*HI-F fragment alone carried in pRPSB104 gives no activity against *crtB4*.

insert would be expected to interrupt transcription. However, we cannot be certain that *Bam*HI-F contains a termination signal, or that it does not supply a new promoter. Restriction mapping of pRPSB105 revealed that the end of the BamG fragment nearest the *Eco*RI-R internal fragment was joined to the Tc^r promoter region; therefore, transcription of the *crtB* gene probably proceeds from left to right on the genetic map as drawn in Fig. 2. Restriction mapping of pRPSB31, which carries both *Bam*HI-G and *Bam*HI-E and gives recombination but no complementation with *crtB4*, showed the *Bam*HI-G fragment to be inverted relative to pRPS105 and separated from the Tc^r promoter by *Bam*HI-E.

The *Bam*HI-I fragment complements the *bchE604* lesion in either orientation of insertion in pDPT42; therefore, we conclude that this restriction fragment carries an *R. capsulata* promoter. Complementation appears to be stronger (more pigment synthesis) in one orientation than in the other. If we assume that vector and insert promoters are in opposite orientations in the plasmid that gives the weaker complementation, the direction of transcription initiated from the chromosomal promoter would be from right to left as drawn in Fig. 2.

Alignment of genetic and restriction maps. The data of Table 3 give the right-left orientation of the genetic map relative to the restriction map, and the scale factor relating genetic map units to kilobases can also be estimated from these data. As presented in Fig. 2, the left end of *Bam*HI-G must be to the left of *crtB4*, because pRPSB105, which contains *Bam*HI-G, complements *crtB4*. The right end of *Bam*HI-J must be to the right of *bchC1007*, because pRPSB40 recombines with *bchC1007*. These genetic markers are separated by 1.46 map units, and the length of DNA indicated is 6.9 kb, which leads to a value of 4.7 kb per map unit as a maximum. Conversely, the right end of *Eco*RI-Q must be to the left of *rxcA142*, because *rxcA142* recombines with DNA from *Eco*RI-B in plasmid pRPSEB2. The left end of the *Bam*HI-C fragment must lie to the right of *bchC1007*, because that mutation is carried by the adjacent piece. The latter observations lead to a value of 4.3 kb per map unit as a minimum. From these considerations we take

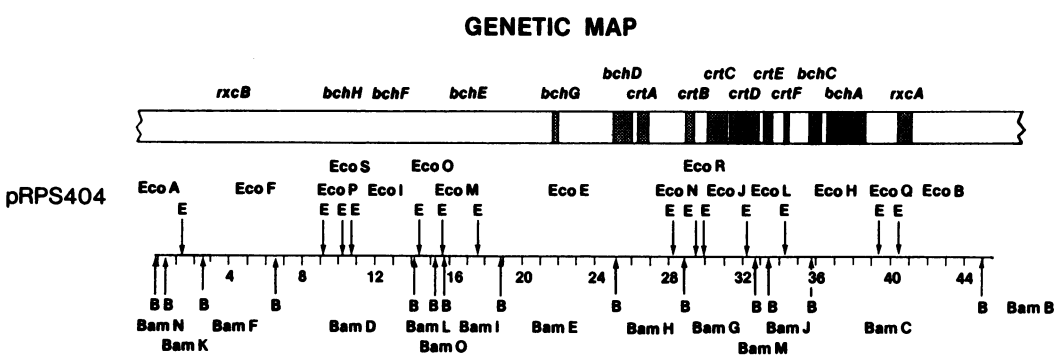

FIG. 2. Alignment of the genetic and restriction maps of the region of the *R. capsulata* chromosome coding for the photosynthetic apparatus. The *bch* genes affect bacteriochlorophyll synthesis, *crt* genes affect carotenoid synthesis, and *rxc* genes affect reaction center synthesis. The shaded areas indicate the genetically determined map positions of groups of mutations conferring the same phenotype. The positions of genes for which no shaded area is indicated were determined by marker rescue, but they have not yet been mapped by genetic techniques capable of precise positioning. The 46 kb of *R. capsulata* DNA carried in pRPS404 is indicated on the restriction map. Arrows labeled E or B indicate the sites of digestion by *Eco*RI or *Bam*HI, respectively. The fragments produced by digestion with either enzyme are named alphabetically by size. The junction fragments, *Eco*RI-A and *Eco*RI-B and *Bam*HI-A and *Bam*HI-B, carry regions of the R-factor that are not indicated on this map (see Clark et al. [submitted for publication] for the complete R-prime structure). *Eco*RI-C, *Eco*RI-G and *Eco*RI-K are comprised entirely of DNA from the vector, pBLM2.

the value of 4.5 kb (3.0 megadaltons) per map unit as the best scale factor for aligning the two maps.

Genetic mapping of *rxcA* and *bchG* loci. When the *rxcA* and *bchG* loci were located on the restriction map, it became feasible to try to fix their positions genetically using so-called ratio test crosses (24). These tests measure the co-transfer between two mutations that confer the same phenotype by measuring the decrease in wild-type recombinants induced by mutant gene donors compared with wild-type donors. The data in Table 4 were used to position the *rxcA* and *bchG* markers on the genetic map.

DISCUSSION

The isolation of R-prime plasmids bearing the *R. capsulata* genes for the photosynthetic apparatus made possible the cloning and restriction enzyme mapping of those genes. To determine the genetic content of the restriction fragments, the marker rescue technique described here was developed. The cloning vehicles pDPT42 and pDPT44 were derived from pBR322 by the addition of a kanamycin resistance gene, a construction necessitated by the lack of markers on pBR322 that functioned well in *R. capsulata*. These vectors retain the property of being amplifiable by chloramphenicol in *E. coli*. pDPT42 has sites suitable for cloning *Bam*HI, *Xho*I, and *Pst*I restriction fragments, and pDPT44 can be used with *Eco*RI, *Sal*I, *Xho*I, *Kpn*I, *Hin*dIII, and *Pst*I (1, 2, 8). The promiscuous mobilizing plasmids pDPT51 and pDPT55 were constructed to mobilize pBR322 and its derivatives from *E. coli* into *R. capsulata*, because *R. capsulata* is not easily transformable. This system for mobilization should be capable of similar applications for all of the hosts in the wide range of the parental plasmid, R751.

Ditta et al. (6) have developed a binary-vehicle, broad-host-range cloning system based upon plasmid RK2, which can be compared to the system described here. They separated the transfer and replication regions of RK2 onto two different plasmids. One, pRK290, retains the parental replication system and serves as a cloning vehicle that is stably maintained in a wide variety of hosts. The other, pRK2013, has the RK2 transfer system and Km^r marker fused to the ColE1 replication system and serves as a helper for mobilization of pRK290. Although the pRK290 system achieves very high frequencies of intergeneric transfer, it was not suitable for the studies reported here because the Km^r marker is on the mobilizing plasmid rather than on the cloning vehicle. An important advantage of our mobilization system is the use of pBR322 vectors, which are widely available and well characterized and can be amplified by chloramphenicol to give high yields of recombinant DNA molecules. pRK2013 does not mobilize pBR322 at

TABLE 4. Genetic mapping of *rxcA* and *bchG* markers[a]

Strains		Psg markers (donor × recipient)	Psg$^+$/Rif^r	ϕ^c	d[d]
Donor	Recipient				
BPY612	BPY27	*bchD561* × *bchG527*	0.784	0.066	0.74
BPY612	BRP33	*bchD561* × *bchG633*	0.907	0.096	0.69
Y402	BPY27	+[e] × *bchG527*	0.839		
Y402	BRP33	+ × *bchG633*	1.003		
BPY381	Y5	*bchA538* × *rxcA5*	0.522	0.159	0.60
BPY381	Y9	*bchA538* × *rxcA9*	0.594	0.173	0.58
BPY381	PAS108	*bchA538* × *rxcA108*	0.686	0.334	0.42
BPY381	Y142	*bchA538* × *rxcA142*	0.622	0.359	0.40
Y402	Y5	+ × *rxcA5*	0.621		
Y402	Y9	+ × *rxcA9*	0.718		
Y402	PAS108	+ × *rxcA108*	1.030		
Y402	Y142	+ × *rxcA142*	0.971		

[a] Gene transfer agent from each donor was used to induce recombinants as previously described (24).
[b] Psg$^+$/Rif^r, Ratio of photosynthetically competent recombinants to rifampin-resistant recombinants measured in the indicated crosses.
[c] ϕ, Cotransfer frequency between the Psg markers calculated as previously described (24).
[d] *d*, Distance between the indicated Psg markers calculated from the map function $d = 1 - \phi^{1/2}$. The map positions of *bchA538* and *bchD561* have been established by previous gene transfer agent-mediated crosses.
[e] +, Wild type.

useful frequencies, nor does pDPT51 mobilize pRK290 (P. A. Scolnik, personal communication).

The mutation (*hsd-1*) that improves the efficiency of *R. capsulata* as a recipient for DNA originating in *E. coli* is reminiscent of a host restriction deficiency; however, a preliminary assay did not reveal a typical restriction endonuclease activity in BY1651. Although mutants bearing *hsd-1* give greater yields of exconjugates in intergeneric crosses, our technique for detecting marker rescue only requires a few colonies that have acquired a given recombinant plasmid, so it was not essential to use *hsd-1* bearing strains for all test crosses.

The alignment of the genetic and restriction maps makes possible the identification of the cloned fragments coding for genes of interest. For example, it seems likely that the *rxcA* or *rxcB* gene (or both) codes for the structural elements in or near the photochemical reaction center in the intracytoplasmic membrane, and sequence information about those important proteins will be useful for studies on reaction center structure and function. The phenotypes of three of the mutants (Y5, Y9, and Y142) with *rxcA* lesions and the one *rxcB* mutant (M124) are the same as that of the classical reaction center-less mutant described in *R. sphaeroides* (4), in which the three reaction center polypeptides and the light-harvesting I antenna complex are all missing. Mutant PAS108 has an unusual phenotype in which reaction center polypeptides are present, but the antenna polypeptide composition resembles that of the other *rxcA* mutants. PAS108 is incapable of photosynthetic growth. Both of these phenotypes are caused by point mutations, and the lesion in PAS108 maps between *rxcA142* and *rxcA9*. The study of mutants with lesions in this region should help to reveal the basis for these pleiotropic changes, but at this time it is not possible to identify the gene product of *rxcA* or *rxcB*.

The scale factor of 3.0 megadaltons per map unit, which was deduced from the alignment of the restriction and genetic maps, is the same as that predicted by an interpretation of the map function: map distance = 1 − (cotransfer frequency)$^{1/2}$. This map function was derived from first principles by assuming that 1 map unit was equivalent to the DNA contained in a single gene transfer agent particle (24). The size of DNA molecules isolated from GTA particles was determined to be about 3 megadaltons (23). The agreement between these disparate approaches to determining the physical correlates of genetic data is gratifying.

This work affords two approaches to an analysis of the pattern of transcription of the genes in this region. First, complementation observed in certain marker rescue crosses gives the direction of transcription of the cloned fragment when

initiation is from a vector-borne promoter of known orientation. Restriction fragments that carry *R. capsulata* promoters include *Bam*HI-I and probably the EB2 fragment, since there is no apparent vector promoter nearby the cloning site on either pRPSB9 or pRPSEB2. These fragments thus bear *R. capsulata* transcription control sites and are of obvious future interest. Complementation is observed for all *bchA* and *bchC* mutants tested with pRPSE30 and the *rxcB* mutants tested with pRPSE2. The results may be attributed to either insert-borne promotors or promoter P1 of pBR322 (18). Promoter P1 has been deleted in pRPSEB2.

One unexpected result is the complementation of *bchD1008* by the *Bam*HI-H fragment of pRPS404, since the same fragment fails to complement or recombine with *bchD561*. If these markers actually lie in the same gene, the simplest expectation would be for both to behave the same in complementation tests. It may be that they are in two separate genes. If they do lie in the same gene, negative complementation may explain the lack of complementation with *bchD561*, but then some wild-type recombinants would be expected. An alternative hypothesis is that *Bam*HI-H carries most, but not all, of *bchD*, and the polypeptide fragment produced can complement one mutation but not the other. The observation that *bchD561* is not rescued by either *Bam*HI-E or *Bam*HI-H suggests that there might be a tiny *Bam*HI fragment missing between *Bam*HI-E and *Bam*HI-H. More mutations in the *bchD* gene(s) are clearly needed to test these hypotheses.

A second avenue of transcription research opened by this study is that of kinetic analysis of mRNA synthesis during repression and derepression of the photosynthetic apparatus, by using RNA-DNA hybridization with the genetically defined restriction fragments. This work is in progress.

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