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Construction of a gene bank of *Rhodopseudomonas capsulata* using a broad host range DNA cloning system *

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Abstract. A gene bank of the phototrophic bacterium *Rhodopseudomonas capsulata* was constructed using the binary plasmid system pRK290/pRK2013. Fragments of about 20 kb of chromosomal DNA of *R. capsulata* strain 37b4 were inserted into the cloning vector pRK290. The hybrid plasmids of the gene bank, maintained in *Escherichia coli* HB101 were transferred by conjugation to *R. capsulata* strains defective in the photosynthetic apparatus with frequencies of 5×10^{-4} to 5×10^{-2}. Phototrophically growing transconjugants occurred with frequencies of 5×10^{-7} to 5×10^{-6}. Recombination between the hybrid plasmids and the *R. capsulata* chromosome was shown. The hybrid plasmid pRCF1002, carrying a 25 kb insert of *R. capsulata* wild type DNA, was isolated from one *E. coli* clone of the gene bank. It reconstituted some bacteriochlorophyll- and photosynthetic negative mutants to phototrophic growth.

Key words: Gene bank – Plasmids pRK290 – pRK2013 – *Rhodopseudomonas capsulata* – Reconstitution – Phototroph negative mutants – Absorption spectra – Light harvesting complexes

Some members of phototrophic bacteria are able to produce ATP alternatively either by photophosphorylation under anaerobic conditions or by oxidative respiratory phosphorylation in the dark in presence of oxygen. Oxygen partial pressure and light intensity are the major external factors which govern the process of membrane differentiation adapting the cells to the respective mode of energy production (reviewed in Drews and Oelze 1981; Ohad and Drews 1982).

After lowering of oxygen tension the synthesis of bacteriochlorophyll *a* and carotenoids as well as of pigment-binding polypeptides is induced. The pigments and the polypeptides assemble in the membrane system to form the photochemical reaction center and the two light-harvesting complexes B870 and B800–850. Reaction center (RC) containing three polypeptides of apparent Mr 28,000, 24,000 and 20,500 and antenna complex B870 (LH I) having two polypeptides of apparent Mr 12,000 and 7,500 are formed simultaneously in *Rhodopseudomonas capsulata* with the same kinetics, while the light-harvesting complex B800–850 (LH II), containing three polypeptides of apparent Mr 14,000, 10,000 and 8,000, assembles independently (Lampe and Drews 1972; Nieth and Drews 1974; Schumacher and Drews 1978, 1979; Dierstein et al. 1981).

The genes coding for bacteriochlorophyll *a* and carotenoid synthesis and for the polypeptides of RC and LH I have been localized on the chromosome of *R. capsulata* (Yen and Marrs 1976; Taylor et al. 1983; Youvan et al. 1984). The R-prime plasmid pRPS404 containing 46 kb chromosomal DNA of *R. capsulata* with the above mentioned "photosynthetic" genes has been isolated by Marrs (1981). The plasmid pRPS404 can be transferred from *Escherichia coli* to *R. capsulata*. It recombines with the *R. capsulata* genome and reconstitutes lesions in mutants blocked in Bchl or carotenoid synthesis and in expression of RC and LH I (Youvan et al. 1982; Taylor et al. 1983).

In this work we describe the construction of a gene bank of *R. capsulata* using a broad host range DNA cloning system developed by Ditta et al. (1980) from the antibiotic resistant plasmid RK2 (Thomas 1982). The cloning vector is the plasmid pRK290 (Tc^r) containing single recognition sites for restriction endonucleases *Eco*RI and *Bgl*II suitable for cloning. Mobilization is effected by the helper plasmid pRK2013 which contains the RK2 *tra* functions (Figurski and Helinski 1979). *Eco*RI fragments of *R. capsulata* DNA of about 20 kb size were inserted into the vector pRK290. The resulting gene bank was transformed to *E. coli* and conjugated to mutant recipient strains. Our major objective was the cloning and detection of further genes encoding functions necessary for pigment complexes of the photosynthetic apparatus besides those already characterized by Marrs (1981). One of the clones with capacity to reconstitute mutants was isolated from *E. coli* and characterized by restriction analysis, hybridization against the plasmid DNA pRPS404 and conjugation with further recipient strains.

Materials and methods

Bacterial strains, plasmids, and culture conditions

Bacterial strains and plasmids utilized in this study are listed in Table 1.

Escherichia coli strains were grown in Luria broth LB (Miller 1972). Strains of *Rhodopseudomonas capsulata* were

* Dedicated to Hans-Günter Schlegel on occasion of his 60th birthday

Abbreviations. Bchl, Bacteriochlorophyll; RC, reaction center; LH, light-harvesting complex; Crt, carotenoid; pho, phototrophic growth; P, Bchl precursor excreted, the number behind P indicates the maximum of absorption in ether (nm); SDS, sodium dodecyl sulfate; Tc, tetracycline; Km, kanamycin; Gm, gentamicin; r, resistant; kb, kilo base pairs

Table 1. Bacterial strains and plasmids

Strain	Genotype/phenotype	Reference/source
Escherichia coli		
HB101	*pro*$^-$, *leu*$^-$, *thr*$^-$, *lacY*$^-$, *hsdM*$^-$, *hsdR*$^-$, *recA*$^-$, *Sm*^{r}	Boyer and Roulland-Dussoix (1969)
BEC404	HB101 (pRPS404), Km^r	Marrs (1981)
Rhodopseudomonas capsulata		
37b4	wild type	German Collection of Microorganisms, Göttingen DSM 938
Ala$^+$pho$^-$	Bchl$^+$, RC$^-$, LHI$^-$, LHII$^-$, Crt$^-$	Drews et al. (1979)
Fm65	Bchl$^-$, RC$^-$, LHI$^-$, LHII$^-$, Crt$^+$, P623	Inst. Biol. 2, Univ. Freiburg, N. Kaufmann
G1	Bchl$^-$, RC$^-$, LHI$^+$, LHII$^-$, Crt$^-$, P653	Inst. Biol. 2, Univ. Freiburg, N. Gadeon
G2	Bchl$^-$, RC$^-$, LHI$^-$, LHII$^-$, Crt$^+$, P653	Inst. Biol. 2, Univ. Freiburg, N. Gadeon
37b4pho$^-$	Bchl$^-$, RC$^-$, LHI$^-$, LHII$^-$, Crt$^+$	Inst. Biol. 2, Univ. Freiburg, N. Gadeon
YS	Bchl$^-$, RC$^-$, LHI$^-$, LHII$^-$, Crt$^+$	Drews et al. (1976)
Y5	Bchl$^+$, RC$^-$, LHI$^-$, LHII$^+$, Crt$^+$	Feick and Drews (1978), Taylor et al. (1983)
GK2	Bchl$^+$, RC$^+$, LHI$^+$, LHII$^-$, Crt$^-$, Tc^r	this work
Glpho$^+$	Bchl$^+$, RC$^+$, LHI$^+$, LHII$^-$, Crt$^-$, Tc^r	this work
plasmids pRK290	Tc^r	Ditta et al. (1980)
pRK2013	Km^r	Figurski and Helinski (1979)
pRPS404	Km^r, bears genes of Bchl, Crt synthesis and RC and LHI	Marrs (1981)
pPHIJI	Gm^r	Beringer et al. (1978)
pRCF1002	Tc^r	this work

grown either chemotrophically or phototrophically in a malate salt medium supplemented with 0.05% yeast extract Difco (Drews 1965). Conjugational matings were carried out on PY plates (1% caseinpeptone, Merck, Darmstadt, FRG, 0.05% yeast extract, Difco, Detroit, MI, USA, 2 mM $MgCl_2$, 2 mM $CaCl_2$, 40 µM $FeSO_4$, 1.5% Difco bacto agar). Tetracycline was used at 20 µg/ml for selection of resistant *E. coli* and at 1 µg/ml to select resistant *R. capsulata* colonies. The concentration of kanamycin for all strains was 25 µg/ml, and of gentamicin 10 µg/ml, respectively. Restriction endonucleases, T4 DNA ligase, and bacterial alkaline phosphatase were purchased from Boehringer, Mannheim, FRG, and used according to suppliers instructions.

Construction of a gene bank of Rhodopseudomonas capsulata

A gene bank of *R. capsulata* strain 37b4 in plasmid pRK290 was constructed according to Ditta et al. (1980). A procedure modified from Birnboim and Doly (1979) was used for the preparation of plasmid DNA. Total DNA from *R. capsulata* was obtained from 800 ml stationary phase grown cells in malate medium. Washed cells were resuspended in 75 mM Tris-HCl buffer, 20 mM EDTA, 100 mM NaCl, pH 8, incubated with 50 mg/ml lysozyme for 30 min at 37° C and afterwards treated with sarcosyl (final concentration 2%) and proteinase K (0.6 mg/ml) for 30 min at the same temperature. An equal volume phenol saturated with 100 mM Tris-HCl buffer pH 8.0 was added and the mixture was shaken vigorously for 30 min at room temperature. The mixture was centrifuged (10,000 rpm, 30 min), and the aqueous phase was extracted three times with phenol/chloroform and twice with chloroform. The nucleic acid was precipitated with ethanol at −20° C. Genomic DNA of *R. capsulata* was partially cut with 2 units of restriction endonuclease *Eco*RI per µg DNA for 2−4 h. Size classes of DNA were separated by sucrose gradient centrifugation (Maniatis et al. 1978). Fractions were monitored for DNA size on a 0.5% agarose gel. Plasmid pRK290 was completely digested with *Eco*RI and treated twice with alkaline phosphatase for 1.5 h at 55° C (Ullrich et al. 1977) to avoid a rejoining of the vector DNA. Fragments of genomic DNA of *R. capsulata* with a size of about 20 kb were ligated into the vector and transformed to *E. coli* by the method of Mandel and Higa (1970). From the transformants tetracycline resistant colonies were selected, resuspended and grown in Luria broth, supplemented with tetracycline to establish the gene bank.

Bacterial matings

The hybrid plasmids of the gene bank and the helper plasmid pRK2013 were simultaneously transferred to *R. capsulata* pho$^-$ mutant strains. Two different methods were used for the trimaternale conjugations. i) 10^8 to 10^9 cells of each of the donor and recipient strains were mixed, harvested by centrifugation, resuspended in a small volume of malate medium and layered onto membrane filters (Sartorius, Type SM, pore size 0.45 µm). The filters were incubated at 32° C on PY agar for 4−7 h, before the cells were resuspended and plated on selective agar. ii) *E. coli* HB101 cells, carrying the hybrid plasmids of the gene bank, were diluted and spread on LB agar plates to obtain single colonies. Lawns of each of the donor strain HB101 (pRK2013) and the recipient strain were grown on LB selective agar and on PY agar plates, respectively. The two donor strains were one after another replicaplated onto the recipient strain and the

PY agar plates were incubated at 32° C for 4–7 h. Finally the colonies were replicaplated on selective agar and incubated under chemotrophic (aerobic) or phototrophic (anaerobic) conditions.

Analysis of nucleic acids

For electrophoretic analysis DNA probes were diluted in TBE buffer (90 mM Tris/HCl, 90 mM borate, 2.5 mM EDTA, pH 8.3), 4% (w/v) sucrose, 0.04% (w/v) bromphenol blue. Electrophoresis was carried out in a horizontal apparatus in TBE buffer (6 V/cm) for 6 h. Fragments of plasmids were separated on 0.8% (w/v) agarose gels. For examination of fragments of chromosomal DNA the agarose concentration was reduced to 0.5%. DNA fragments were blotted to nitrocellulose BA 85 (Schleicher and Schüll, Dassel, FRG) as described (Southern 1975).

Plasmids were labeled with ^{32}P by nick translation (Maniatis et al. 1975; Rigby et al. 1977). Hybridization was performed as previously described (Wahl 1979; Thomas 1980).

Spectral analysis of R. capsulata strains

20–40 ml of cells from stationary growth phase were harvested (8,000 rpm, 10 min) and resuspended in 5 ml of 30 mM Tris-HCl buffer, 5 mM EDTA, pH 8.0. The suspension was frozen in liquid nitrogen, thawed at room temperature and sonicated five times for periods of 15 s with interruptions of 30 s. After centrifugation (15,000 rpm, 20 min) the absorption spectra of the supernatant containing the intracytoplasmic membrane fraction were recorded on a Kontron Uvikon spectrophotometer, type 810.

Electrophoretic analysis of proteins

Membrane proteins were separated by SDS polyacrylamide gel electrophoresis on 1 mm slab gels (Laemmli 1970) using a 11.5–16.5% continuous gradient of acrylamide. Preparation of crude membranes has been described previously (Feick and Drews 1978).

Results

Construction of a gene bank from R. capsulata DNA

Size-fractionated *R. capsulata* DNA was ligated to the vector plasmid pRK290 and used to transform *E. coli* strain HB101 to tetracycline resistance. The mean size of the DNA inserts was determined by agarose gel electrophoresis to be approximately 20 kb. 2,200 Tc resistant colonies of *E. coli* HB101 transformants were taken to establish a gene bank of *R. capsulata*. Test ligation was carried out with alkaline phosphatase treated *Eco*RI digested pRK290 without chromosomal DNA. Transformation to *E. coli* HB101 resulted in a number of Tc resistant colonies which was only 5–10% of the number of Tc resistant colonies obtained with pRK290 containing chromosomal DNA inserts. These results suggest that about 2,000 transformants of *E. coli* HB101 contained inserts of chromosomal DNA of *R. capsulata* within their plasmids. The chromosome of *R. capsulata* is assumed to consist of 42,000 kb (Yen and Marrs 1976). From this value and the mean insert size of 20 kb there is a chance of 99% that a given sequence of *R. capsulata* DNA is present in the gene bank.

Matings with the gene bank

The gene bank was tested with *R. capsulata* mutant strains, defective in the photosynthetic apparatus and unable to grow phototrophically. The gene bank (*E. coli* HB101 containing pRK290 with chromosomal inserts) and *E. coli* HB101 with the helper plasmid pRK2013 were crossed with the mutant strains on filters as described under methods. The frequency of conjugational transfer calculated from the number of Tc-resistant chemotrophically growing transconjugants was 5×10^{-4} to 5×10^{-2} (Table 2). The pho$^-$ mutant strain Fm65 was reconstituted to wild type with a frequency of 5×10^{-7} and the strain G1 was complemented to green phototroph positive colonies with a frequency of $5-10^{-6}$ per recipient. In contrast to the behavior of the wild type strain the G1 pho$^+$ transconjugant showed absorption spectra which differ strongly whether the culture was grown anaerobically in the light or semiaerobically in the dark (Fig. 1). The phototrophic positiv transconjugant G1 pho$^+$ formed polypeptides of RC and both light-harvesting complexes which are missed in the recipient strain (Fig. 3), but the light-harvesting complex B800–850 was not expressed. Plasmid DNA, isolated from reconstituted strains, was transformed to *E. coli* HB101 and transferred by conjugation to the respective mutant strain (Table 2B). The frequency of reconstitution to phototrophic growth increased 10 to 500 times compared to reconstitution with the gene bank.

Chromosomal integration of the transferred DNA

In order to study the fate of the chromosomal DNA in the hybrid plasmid after transfer from the *E. coli* donor to the *R. capsulata* recipient strain the reconstituted *R. capsulata* strains were mated with *E. coli* strain HB101 (pPH1JI) (Table 2, E). The plasmid pPH1JI confers gentamicin resistance and belongs to the same incompatibility group as pRK290 (Beringer et al. 1978). Consequently transconjugants which were Gm and Tc resistant could not be established and have not been detected. Gm resistant and Tc sensitive conjugants of *R. capsulata* retained the capacity of phototrophic growth for many generations. From these observations it is tentatively concluded that genes on the hybrid plasmid encoding phototrophic functions were integrated in the chromosome of the *R. capsulata* mutants by recombination and that the plasmid pRK290 was displaced from *R. capsulata* cells by the plasmid pPH1JI.

Isolation of plasmids containing genes of pigment complex formation

Because the hybrid plasmids showed instability in *R. capsulata* due to recombination, we tried to isolate those plasmids from *E. coli* clones, which contained the capacity to reconstitute phototroph negative mutants of *R. capsulata* to phototrophic growth. For this purpose single colonies of the gene bank were tested for complementation of *R. capsulata* mutant strains by the replica plating method described under methods. Reconstitution-positive clones were selected for mating with the same recipients using the filter technique. The frequencies of reconstitution corre-

Table 2. Frequencies of conjugation and reconstitution of *Rhodopseudomonas capsulata* mutant strains

Donor *E. coli*	Recipient *R. capsulata*	Frequency of conjugation per recipient	Number of phototrophically growing colonies per recipient	Phenotype of phototrophic transconjugants	Designation of transconjugant strains
A					
gene bank	Fm65	5×10^{-4}	5×10^{-7}	wild type	Fm65pho$^+$
gene bank	G1	10^{-2}	5×10^{-6}	Bchl$^+$, RC$^+$, LHI$^+$, LHII$^-$, Crt$^-$	Glpho$^+$
gene bank	G2	10^{-2}	2×10^{-6}	red colonies, wild type, excreeting pigment, and green colonies identical with Glpho$^+$	G2pho$^+$
B					
HB101 (pFm65pho$^+$)	Fm65	5×10^{-4}	10^{-5}	wild type	
HB101 (pGlpho$^+$)	G1	10^{-2}	9×10^{-5}	Glpho$^+$ phenotype	
HB101 (pG2pho$^+$)	G2	10^{-2}	5×10^{-5}	G2pho$^+$ phenotype	
HB101 (pG2pho$^+$)	G1	10^{-2}	2×10^{-5}	Glpho$^+$ and G2pho$^+$ phenotype	
C					
HB101 (pRCF1002)	Ala$^+$pho$^-$	2×10^{-3}	2×10^{-3}	Bchl$^+$, RC$^+$, LHI$^+$, LHII$^-$, Crt$^-$	GK2
HB101 (pRCF1002)	37b4pho$^-$	8×10^{-3}	5×10^{-6}	wild type	
HB101 (pRCF1002)	Y5	5×10^{-5}	2×10^{-5}	wild type	
HB101 (pRCF1002)	YS	10^{-3}	5×10^{-5}	Bchl$^+$, RC$^+$, LHI$^+$, LHII$^-$, Crt$^+$	
D					
BEC 404 (pRPS404)	Fm65	5×10^{-3}	5×10^{-6}	yellow and green colonies	
BEC 404 (pRPS404)	G1	8×10^{-3}	10^{-3}	wild type and green colonies	
BEC 404 (pRPS404)	G2	2×10^{-3}	2×10^{-3}	wild type and green colonies	
BEC 404 (pRPS404)	Ala$^+$pho$^-$	10^{-4}	10^{-4}	red and yellow colonies	
BEC 404 (pRPS404)	37b4pho$^-$	10^{-5}	9×10^{-6}	wild type	
BEC 404 (pRPS404)	Y5	2×10^{-3}	2×10^{-3}	wild type and yellow colonies	
BEC 404 (pRPS404)	YS	8×10^{-4}	6×10^{-5}	yellow colonies	
E					
HB101 (pPH1JI)	Fm65pho$^+$	5×10^{-3}	5×10^{-3}	Fm65pho$^+$ phenotype Gm^r Tc^s	
HB101 (pPH1JI)	Glpho$^+$	8×10^{-3}	2×10^{-3}	Glpho$^+$ phenotype	
HB101 (pPH1JI)	G2pho$^+$	8×10^{-3}	8×10^{-3}	G2pho$^+$ phenotype	
HB101 (pPH1JI)	GK2	2×10^{-2}	10^{-2}	GK2 phenotype	

The frequencies of conjugation were calculated from Tc^r or Gen^r colonies growing under chemotrophic conditions. All recipient strains were also mated with HB101 (pRK290) as control for revertants. The matings listed here showed no phototrophic colonies in this control experiment. The indicated frequencies represent the average of 3–4 identical experiments and differed maximal with a factor of 10. Plasmids isolated from phototrophically growing transconjugants of experiment A were transformed into *E. coli* HB101. The transformants were washed off the plates and served as donors in experiment B. Because of the recombinational events, which occurred in the *R. capsulata* transconjugants, the *E. coli* donor cells in experiment B contained a mixture of different pRK290 derivatives (designated with p and the name of the transconjugant, from which the plasmids were isolated). In experiment C the donor cells were grown from a single colony of *E. coli*, so that all of them contained identical plasmids. Phototrophically growing transconjugants showed no change in their phenotype, when the pRK290 derivatives were replaced by the plasmid pPH1JI by conjugation in experiment E. Transconjugants of this experiment were selected for Gm^r

sponded with the frequencies of conjugation (Table 2, C). Here we describe the analysis of *E. coli* HB101 (pRCF1002), which reconstituted the mutant strain Ala$^+$pho$^-$ to slow phototrophic growth. The absorption spectra of the cell-free extracts from Ala$^+$pho$^-$ and the transconjugant GK2 show clear differences in the height and position of peaks (Fig. 2). SDS polyacrylamide gel electrophoresis of the membrane fractions of Ala$^+$pho$^-$ and GK2 revealed, that polypeptides of the pigment-protein complexes were synthesized by the reconstituted strain GK2, which are not detected in the recipient strain Ala$^+$pho$^-$ (Fig. 3f, h). In addition to bands of RC and LHI subunits the 10 k subunit of the complex B800–850 occurred in GK2, though the spectrum showed no typical B800–850 absorption. From *E. coli* HB101 (pRCF1002), which complemented strain Ala$^+$pho$^-$ to phototrophic growth, a 45 kb plasmid was isolated. This pRK290 derivative, pRCF1002, carries an insert of about 25 kb, which has two recognition sites for each of the restriction endonucleases *Xho*I, *Sst*II, one for *Eco*RI, and none for *Bgl*II. In order to show that the insert of pRCF1002 contains chromosomal DNA, the ^{32}P-labeled pRCF1002 was hybridized against chromosomal DNA of *R. capsulata* 37b4 digested with *Eco*RI. On the autoradiogram several *Eco*RI fragments of the chromosomal DNA of *R. capsulata* were detected in addition to the both internal *Eco*RI fragments of pRCF1002 (not shown). When the vector pRK290 was taken as radioactive probe, no signals were found on the autoradiogram using the same hybridization conditions. These results show, that the insert of pRCF1002 derives from chromosomal DNA of *R. capsulata*.

Although pRCF1002 reconstituted mutants with defects in the photosynthetic apparatus the reconstitution patterns

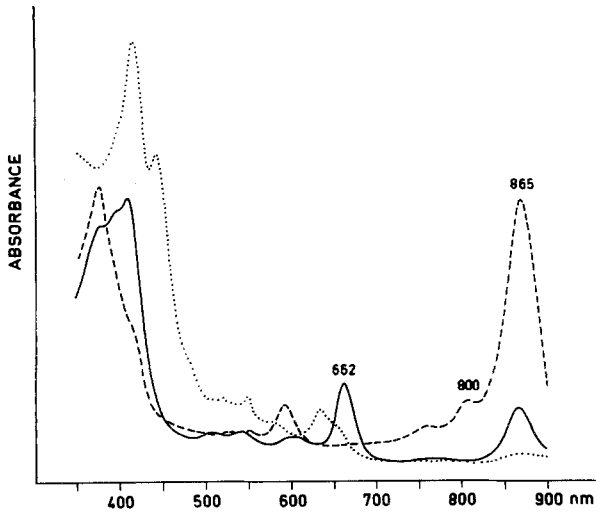

Fig. 1. Absorption spectra of the cell-free extracts of the *Rhodopseudomonas capsulata* mutant strain Gl (——) and the reconstituted strain Glpho$^+$ grown semiaerobically in the dark (· · · ·) and anaerobically in the light (– – – –). The recipient strain Gl and the anaerobically grown reconstitutent Glpho$^+$ show the B870 absorption, which is missing, when Glpho$^+$ is grown semiaerobically. A distinct peak at 372 nm occurs in Glpho$^+$ grown anaerobically, which is masked by an absorption peak at 414 nm, when this strain is cultured semiaerobically

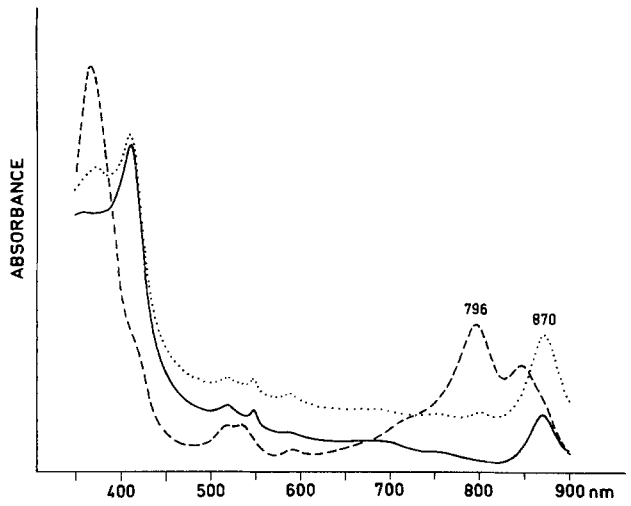

Fig. 2. Absorption spectra of the cell-free extracts of the *Rhodopseudomonas capsulata* mutant strain Ala$^+$pho$^-$ (——) and the reconstituent GK2 grown semiaerobically in the dark (· · · ·) and anaerobically in the light (– – – –). The GK2 strain shows strong absorption in the region of 370 nm. The absorption between 700 and 900 nm is clearly different from the absorption peaks of wild type 37b4 (see Weaver et al. 1975). The typical 800 nm and 855 nm peaks of the wild type are shifted to shorter wavelengths

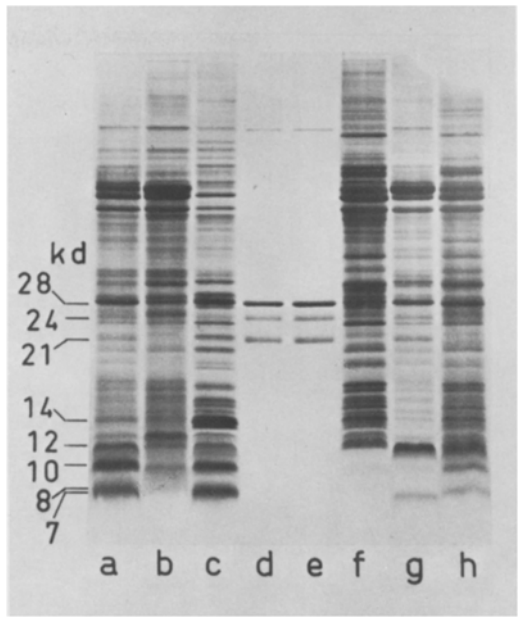

Fig. 3. The protein patterns of the membrane fractions of the mutant strains Gl (lane *b*) and Ala$^+$pho$^-$ (lane *f*) and their reconstituents Glpho$^+$ (lane *a*) and GK2 (lane *h*) visualized by SDS polyacrylamide gel electrophoresis. The apparent M_r of the polypeptides of the RC (28, 24, 21 k) and the pigment-protein complexes (14, 12, 10, 8, 7 k) from wild type *Rhodopseudomonas capsulata* 37b4 (lane *c*) are indicated. Lanes *d* and *e* show the polypeptides of isolated RC complex. The mutant strain Ala$^+$ (lane *g*), having RC and B870 but missing B800–850 and carotenoid absorption has the polypeptides 28, 24, 21, 12, 7 k. In both reconstituted strains, Glpho$^+$ and GK2, polypeptide bands are visible which are absent in the recipient strain. The 10 k polypeptide of LH II (B800–850) complex occurs in both reconstituents, though typical absorption bands of this complex were not found

of pRCF1002 were found to be different from that of pRPS404. Furthermore the insert of the plasmid pRPS404 derived not from *R. capsulata* 37b4. In order to study homology between both plasmids pRCF1002 ^{32}P-labeled by nick translation was hybridized against pRPS404 DNA cut with *Eco*RI and *Eco*RI plus one further restriction endonuclease, respectively. None of the internal *Eco*RI fragments of the chromosomal DNA of pRPS404 showed homology to pRCF1002 (Fig. 4, lane e). Fragments detected by autoradiography belong either to the vector or are composed of vector DNA and of chromosomal DNA of *R. capsulata*. The same negative result was obtained, when *Eco*RI/*Pst*I, *Eco*RI/*Xho*I, or *Eco*RI/*Kpn*I fragments of pRPS404 were used (not shown). When pRCF1002 was hybridized against pRPS404, which was cut with *Eco*RI and *Sst*II, three additional fragments occurred on the autoradiogram (Fig. 4, lane f). Two of these fragments with 2.1 and 2.0 kb derive from the vector RK2. The third fragment of about 1.8 kb is not vector specific when compared to the restriction maps of RK2 (Thomas 1982) and of pRPS404 (Marrs, pers. comm). If the 1.8 kb band results from hybridization between the inserts of pRCF1002 and pRPS404, it is possible, that the homologous, hybridizing sequence is part of one of the *Eco*RI fragments of pRPS404, which are localized at the ends of the insert.

E. coli HB101 (pRCF1002) was mated with other *R. capsulata* mutants. The strains 37b4pho$^-$ and Y5 were reconstituted to wild type. The frequencies of reconstitution corresponded with the frequencies of conjugation or were up to 100 times lower (Table 2, C). After mating of the mutant strain YS phototrophic positive colonies with light green color were detected. The absorption spectrum of the phototrophically grown transconjugant showed peaks similar to the GK2 absorption spectrum (not shown here).

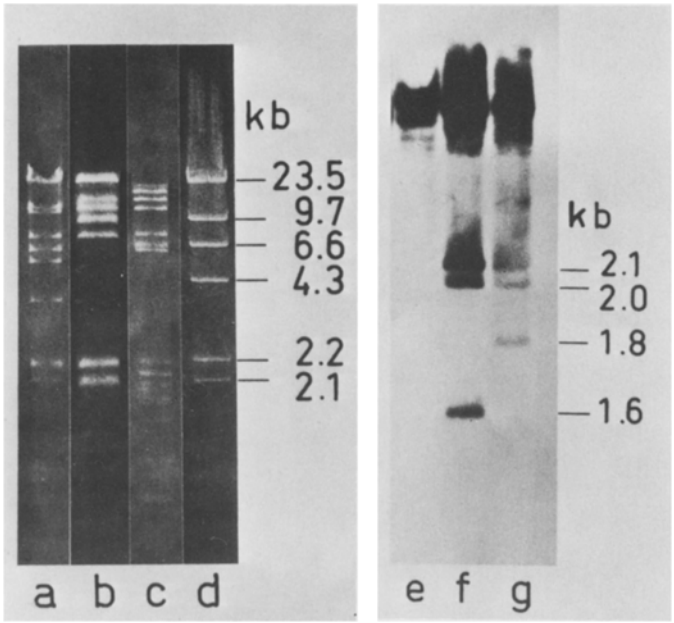

Fig. 4. Agarose gel electrophoresis of pRPS404, DNA cut with *Eco*RI (lane *a*) or cut with *Eco*RI and *Sst*II (lane *c*) and of hybrid plasmid pRCF1002, cut with *Eco*RI and *Sst*II (lane *b*). Marker DNA from the phage λ was cut with *Hind*III (BRL) (lane *d*). The fragments were blotted to nitrocellulose as described and hybridized against ^{32}P labelled pRCF1002. The autoradiogram is shown on lanes *e*–*g*. When pRPS404 DNA was cut with *Eco*RI, only those fragments hybridized, which belong to the vector or which contained vector DNA and chromosomal DNA of *R. capsulata* (lane *e*).. When pRPS404 was cut with *Eco*RI and *Sst*II three additional hybridizing fragments occured (lane *g*). Two of these fragments with 2.1 and 2.0 kb could be derived from the vector RK2 and are identical with two fragments of pRCF1002 belonging to the pRK290 vector (lane *f*). The 1.8 kb fragment occurring on lane *g* is not vector specific taking the restriction maps of RK2 (Thomas 1982) and pRPS404 (Marrs, pers. comm.) as basis

Comparison of transconjugants reconstituted either by pRPS404 or gene bank plasmids

All recipients used in this study were reconstituted to phototrophic growth and wilde type absorption spectrum by mating with the plasmid pRPS404 except the mutant strains Fm65 and YS. Transconjugants of these strains showed yellow or green colored phototrophically grown colonies. No wild type reconstituents were found. The frequencies of conjugation and reconstitution are given in Table 2, D.

Discussion

Fragments of about 20 kb of chromosomal DNA, isolated from *R. capsulata* strain 37b4, were cloned into the broad host range vector pRK920 and established in *E. coli*. Large inserts were used to generate the gene bank in order to increase the probability of preserving functional gene clusters intact. The main intention was to use the gene bank for the screening and analysis of genes encoding structure and synthesis of the photosynthetic apparatus of *Rhodopseudomonas capsulata*. Similar work has been carried out using derived vector systems for complementation studies of the *nif* gene clusters and other markers in the genus of *Rhizobium* (Friedmann et al. 1982, Ruvkun and Ausubel 1981). Recombination of cloned DNA and the chromosome of *R. capsulata* mutant strains was described in this work. One of the hybrid plasmids of the gene bank reconstituted different photosynthetic negative mutants to phototrophic growth. Studying gene expression by reconstitution one has to be aware that the formation of the pigment-protein complexes is a process regulated on different levels. The complementation of Bchl biosynthesis might be a prerequisite of an ordered assembly process into the membrane. The 10 k polypeptide of B800–850 complex has a fast turnover, when Bchl is not synthesized in the mutant strain YS (Dierstein 1983). The idea that complex formation is partially reconstituted, if Bchl synthesis is restored was supported by conjugation experiments with *E. coli* HB101 (pRCF1002) (Table 2). In all phototrophic transconjugants RC and B870 were reconstituted, whereas carotinoids and B800–850 were not. Some polypeptide subunits were formed without concomittant synthesis of the respective protein-pigment complexes. Earlier studies in our laboratory showed that mutants, which are phenotypically defect in the formation of the pigment complexes, often are able to synthesize single proteinous components of the complexes (M. Braun, R. Dierstein, N. Kaufmann, M. Tadros, unpublished).

Considering the 1.8 kb homologous fragment, which was shared by pRCF1002 and pRPS404, it has to be studied further, whether both plasmids have overlapping or adjoining gene sequence, because chromosomal inserts of both plasmids were able to complement defects in synthesis of Bchl and Bchl-binding proteins. Experiments using pRPS404 as radioactive probe for hybridization against chromosomal DNA from different *R. capsulata* strains have shown different hybridization patterns in the "photosynthetic region" *R. capsulata* strain St. Louis (parent strain of the pRPS404 insert) and strain 37b4 (parent strain for the insert of pRCF1002) (E. Lübke, unpublished). These results point to differences in the gene maps of the both strains, which render it possible that identical genes carry different recognition sites for restriction endonucleases complicating the search for homologous sequences.

The R-prime pRPS404, which was the first hybrid plasmid containing *R. capsulata* DNA encoding the photosynthetic apparatus, was obtained from chromosomal DNA of the strain St. Louis by chromosomal mobilization into the vector pBLM2. The chromosomal insert in pRPS404 is flanked by two IS21 elements. Recombination between pRPS404 and the *R. capsulata* chromosome is due to recombination of IS sequences (Youvan et al. 1982). In this work chromosomal DNA of strain 37b4 was inserted at a single recognition site for *Eco*RI into the vector. Thus recombination with the chromosome of mutant strains cannot be explained by recombination of IS elements.

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