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Refinement of the high-resolution physical and genetic map of *Rhodobacter capsulatus* and genome surveys using blots of the cosmid encyclopedia

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Cosmids from a library containing *Rhodobacter capsulatus* DNA fragments were previously ordered in two contigs: one corresponding to the chromosome and one to a 134 kb plasmid. This map contained 40 regions connected only by colony hybridization. To confirm the linkage and correct the map, the actual sizes of the overlaps were determined by blot-hybridization with *Rhodobacter* chromosomal DNA and by mapping of additional cosmids. Several revisions of the earlier map include single cosmid shifts and inversions. One additional gap in a cosmid contig was also found, raising the possibility that the chromosome is not a contiguous circle. About 2500 additional *Eco*RI, *Bam*HI and *Hin*dIII restriction sites were added to the 560 *Eco*RV sites previously mapped onto the *Rhodobacter* chromosome, increasing the resolution of the physical map to the size of individual genes. Twenty-five new markers were located on the genetic map. The 48 markers now mapped represent nearly 300 genes and ORFs cloned from different species of *Rhodobacter*. The orientation of transcription of the four *rrn* operons was established using 16S rRNA- and 23S rRNA-specific probes and digestion with the rare-cutting enzyme, *Ceu*I. Gel blots of 192 cosmids of the miniset of *R.capsulatus* digested with *Eco*RV were prepared. Such a hybridization template represents the whole genome cut into 560 DNA fragments varying in size from 0.4 to 25 kb. This template was used for high-resolution mapping of single genes, analysis of total genomic DNAs from related *Rhodobacter* strains and differentially expressed RNAs.

Key words: cosmid encyclopedia/genome mapping/hybridization template/*Rhodobacter capsulatus*

Introduction

Rhodobacter capsulatus is a purple, non-sulfur photosynthetic bacterium. The biochemical versatility to choose between photo- and heterotrophic growth and to fix nitrogen is packed into a genome that is smaller than that of *Escherichia coli*. Simplicity of plating and generation of mutations, together with the availability of convenient systems for cloning and genetic analysis (Yen and Marrs, 1976; Yen *et al.*, 1979; Marrs, 1981; Johnson *et al.*, 1986), summarized in Donohue and Kaplan (1991), make *R.capsulatus* a popular model system for studies of the photosynthetic apparatus and nitrogen fixation. About 200 genes involved in these processes have been identified and sequenced. More than 150 GenBank entries associated with this organism and the closely related *R.sphaeroides* can be fused in ~50 clusters ranging from a few base pairs to 45 kb long.

Genetic mapping becomes an essential tool when processes like photosynthesis and nitrogen fixation, involving hundreds of genes with often indistinguishable phenotypes, are studied. Mobilization of the bacterial chromosome by integrated R′ factors produced the current genetic map of *R.capsulatus* (Willison *et al.*, 1985; Willison, 1993).

Physical mapping of bacterial genomes, begun with *E.coli* (Smith *et al.*, 1987), has been applied successfully to nearly a hundred different microorganisms (M.Fonstein and R.Haselkorn, submitted). Blot-hybridization of cloned probes in order to localize them on a restriction map has become an easier alternative then traditional genetic mapping. Physical mapping using 'genome encyclopedias' was first demonstrated in the ordering of a set of λ clones of *E.coli* (Kohara *et al.*, 1987). The successful use of the gene encyclopedia in studies of *E.coli* [~300 new genes added to 1400 genetically mapped (Riley, 1993)] encouraged other studies. Encyclopedias of *Mycoplasma pneumonia* (Wenzel and Herrmann, 1989), *Myxococcus xanthus* (Kuspa *et al.*, 1989), *Haloferax volcanii* (Charlebois *et al.*, 1991), *Bacillus subtilis* (Azevedo *et al.*, 1993), *Mycobacterium leprae* (Eiglmeier *et al.*, 1993), *Helicobacter pilory* (Bukanov and Berg, 1994) and of several *E.coli* strains, have been constructed.

A gene encyclopedia of *R.capsulatus* SB1003 DNA fragments in cosmid Lorist 6 has been assembled (Fonstein and Haselkorn, 1993). One hundred ninety two cosmid clones of this encyclopedia cover the 3.7 Mb chromosome of *R.capsulatus* and its 130 kb plasmid. Twenty-three cloned genes and eight groups of repeated sequences were located on 560 *Eco*RV fragments comprising the entire genome. This resolution, however, was not sufficient for many applications of the encyclopedia, for example, for the mapping of differentially expressed transcripts. In addition, 40 areas remained where overlapping cosmids did not have common restriction fragments.

In the present work, the maps of these 40 regions have been verified and corrected by blot-hybridization. Restriction sites for three enzymes, *Eco*RI, *Bam*HI and *Hin*dIII, and 25 new genetic markers were added to the map. Finally, applications of a blot of the miniset of digested cosmids, termed 'high-resolution hybridization template' (HRHT), for gene mapping, strain comparisons and studies of the global regulatory network (Chuang *et al.*, 1993) are illustrated.

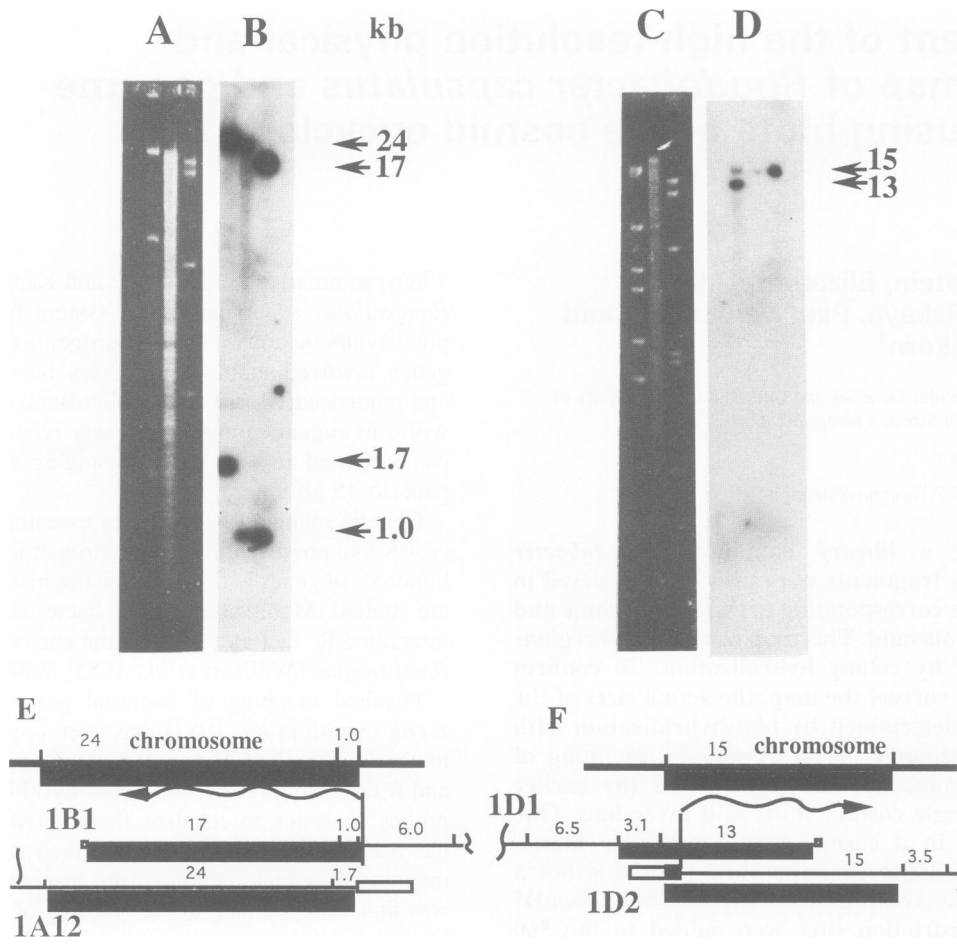

Fig. 1. Linking of 'ambiguous' cosmids by blot-hybridization. (**A**) Cosmid 1A12 DNA, chromosomal DNA of *R.capsulatus* and cosmid 1B1 DNA digested by *Eco*RV. (**B**) Blot-hybridization of these DNAs with a probe generated by SP6 RNA polymerase on the 1A12 template. (**C**) Cosmid 1D1 DNA, chromosomal DNA of *R.capsulatus* and cosmid 1D2 DNA digested by *Eco*RV; (**D**) Blot-hybridization of these DNAs with a probe generated by SP6 RNA polymerase on the 1D2 template (lanes are reversed compared with C). (**E** and **F**) *Eco*RV maps of the overlapping regions: black rectangles represent fragments revealed by hybridization, numbers above the lanes and at the arrows represent the fragment sizes and wavy lines with arrows represent SP6-specific transcripts.

Results

Stapling of the cosmid overlaps

To generate sufficiently random insert ends, *Sau*3A was used for digestion of the *Rhodobacter* chromosome in the construction of the original Lorist 6 library (Fonstein *et al.*, 1992). As a consequence, 40 junctions in the map were linked by cosmids that did not have common *Eco*RV fragments in overlapping regions. This factor added uncertainty to the map due to possible 'slipping' in such places. The sum of the maximum possible shifts was ~150 kb. Blot-hybridizations of the cosmids and chromosomal DNA probed with SP6- or T7-specific transcripts generated from the ends of the inserts of the ambiguous cosmids were used to determine a precise map in these overlapping regions. The results of a typical experiment are shown in Figure 1. Cosmids in Figure 1A, B and E share a common *Eco*RV site, as in 20 of the overlaps tested. Cosmids in Figure 1C, D and F do not have such a site. However, the fact that similar fragments were identified in reciprocal hybridizations both in cosmids and in chromosomal DNA provided evidence of linkage in such cases. When no common *Eco*RV site was found in the overlapping region, the size of the fragment revealed by blot-hybridization in the digest of the chromosomal DNA was used for a final map. Adjustments were required in four regions of the earlier map. In the case of the linkage between cosmids 1G6–1G7, this discrepancy was due to a mistake in the earlier restriction mapping that resulted in the inversion of cosmid 1G6 with respect to its real position. In two other cases, 1B10–1B8 and 2G7–2G6, additional screening of the primary cosmid library and mapping of additional cosmids had to be done to close small gaps. In the case of 1D8–1D9 linkage, their cross-hybridization was due to internal repeats (data not shown), and no really linking clones were found in additional experiments among 1800 new cosmids tested. Finally two gaps, 1D8–1D9 and 1A1–2A12 (the latter linked by hybridization with PFGE-generated fragments *Ase*I−7 and *Xba*I−8 (Fonstein *et al.*, 1992) remained after these analyses. Since the gap between 1A1 and 2A12 can be covered by a single PFGE fragment, the absence of this region from the cosmid and λ libraries suggests that it contains an unclonable sequence. The gap between 1D8 and 1D, on the other hand, may be due to the real absence of a continuity, i.e. that these cosmids contain the ends of a linear chromosome.

Gene mapping

To extend the genetic map of *R.capsulatus*, 25 new genes, listed in Table I, were located on its physical map (Figure 2) using a two-stage scheme described previously (Fonstein and Haselkorn, 1993). Half of them had already been placed with the accuracy of the *Eco*RV restriction map. The resolution of mapping was increased when the four-enzyme map (*Eco*RV, *Eco*RI, *Bam*HI and *Hin*dIII) was constructed. Several probes were kindly provided by R.Tabita, F.Daldal and M.Pollich, or derived from genes cloned in our laboratory. Fifteen probes used in the study were synthesized by PCR using genomic DNA of *R.capsulatus* SB1003 as template. Oligos for PCR were designed according to GenBank entries for different species of *Rhodobacter*. Precise positions of the amplified fragments relative to the beginnings of GenBank entries are listed in Table I. We failed to achieve single PCR products for five genes. Technical errors aside, this could be due to sequence divergence between species. The use of homologous DNA (e.g. *R.sphaeroides*) as PCR template should solve this problem.

Orientation of transcription of the rrn operons

The position of the *rrn* operons and the orientation of their transcription are important features of bacterial genome architecture influencing, for example, genome rearrangements (Hill and Gray, 1988; Fonstein *et al.*, 1994). There are four *rrn* operons located on the chromosome of *R.capsulatus* SB1003 (Fonstein and Haselkorn, 1993). In many genera of microorganisms, the extremely rare-cutting endonuclease *Ceu*I digests *rrn* operons (Liu *et al.*, 1993) and usually these are the only cleavage sites for the whole genome. As in many microorganisms, *rrs* (16S rRNA) is the first gene in the *rrn* operons of *R.capsulatus*. *Ceu*I splits *rrl* (23S rRNA) near the middle (Regensburger *et al.*, 1988). Gel blots of cosmids carrying *rrn* operons digested with *Eco*RV and *Eco*RV+*Ceu*I were probed with PCR-generated fragments of 16S and 23S RNAs. Primers for this amplification were kindly provided by M.McClelland. The order of 16S- and 23S-specific probes in the cosmids determined the orientation of transcription of the operons (Figure 2).

Genome mapping of EcoRI, BamHI and HindIII sites

About 560 *Eco*RV restriction sites were positioned on the 3.7 Mb chromosome of *R.capsulatus* in the previous version of the physical map. This resolution (one site per 6.5 kb) is not sufficient for the comparison of restriction maps derived from sequencing data (usually 2–10 kb long) with the genome map. Some other applications of the genome encyclopedia, such as use as a hybridization template, also depend on its resolution. Restriction sites of *Eco*RI, *Bam*HI and *Hin*dIII were therefore mapped in all 192 cosmids comprising the encyclopedia. The mapping was performed using λ terminase cleavage and partial digestion with each enzyme. The products of the partial digestions were revealed using labeled oligos complementary to the *cosL* or *cosR* site (Figure 3A) and analyzed (Figure 3B and D), as described in Materials and methods. Restriction maps of individual cosmids were merged in three uninterrupted stretches linked with one λ clone and PFGE mapping (Figure 2). More than 3000 restriction sites were mapped. Two-thirds of them were located on at least two overlapping cosmids. Two genome regions, a 9 kb fragment of the *hup* gene cluster (Colbeau *et al.*, 1993) and 17 kb from the photosynthetic gene cluster merged from several GenBank entries, were chosen to check the accuracy of the mapping (Figure 4). This comparison revealed the loss of one 20 bp and one 200 bp fragment and misplacement of two small fragments in the *Eco*RI digest. Twenty-seven fragments of the 29 derived from sequencing data correspond to the ones mapped by terminase within 5% error.

Use of the cosmid set as a high-resolution hybridization template

One hundred ninety two cosmids were digested with *Eco*RV, separated in two agarose gels and transferred onto two 20×25 cm nylon membranes (Figure 5). These blots, called HRHT, are equivalent to Kohara's 'hybridization membranes' of the *E.coli* genome with the following difference: the average *Eco*RV fragment size is 6.5 kb, three times smaller than the inserts in the Kohara library. Several types of probe were used in hybridizations with this HRHT.

Two probes, one corresponding to the *Q* gene and the other to the *P* gene of a permease operon, were used to map this operon on the HRHT. The strong signals produced with both probes positioned these genes in cosmid 2A3 (data not shown). However, ~20 signals (10–20 times weaker) were observed with other members of the cosmid set even at high stringency of hybridization (Figure 6A and B, cosmids 1–96) with both probes. These positive cosmids formed two overlapping but different groups. Presumably, the two probes revealed other operons hybridizing either to the conserved permease domain (*P*), to its ATP-binding protein (*Q*), or in some cases, to both. Decreasing the detectable target for hybridization to a unique restriction fragment increases the sensitivity of the method and allows us to survey the whole genome for the presence of multiple repeated elements in a single experiment with the HRHT. The resolution of the HRHT makes it possible to locate these elements on the map with sufficient accuracy to start sequencing of the areas of interest.

Total chromosomal DNA of *Rhodobacter* strain B1 was used as another probe against the HRHT. DNA of *R.capsulatus* SB1003 served as control. Most of the hybridization signals were proportional to the intensity of the EtBr-stained fragments within 10% error limits. The measurements were done using a Fuji phosphor-imager and compared with the scanned photographs (data not shown). About 10 fragments have 3–10 times higher relative intensity and may correspond to yet unidentified repeated elements. *Eco*RV fragments that carry cosmid vector sequences can be considered as internal negative controls. Only one 10 kb fragment (*Eco*RV−485) was not detected by the B1 probe in repeated experiments. In a more detailed comparative study of different *Rhodobacter* strains (M.Fonstein *et al.*, submitted), B1 was found to be nearly identical to SB1003, which explains our failure to reveal many missing fragments.

RNA samples generated under different physiological conditions were also used as probes against the HRHT, an approach used in Trieselmann and Charlebois (1992).

Table I. Mapped *Rhodobacter* genes

#	gene/cluster	gene mapped	cosmid address **Primer#**	sequenced DNA (nt)	# of genes ORFs	function or corresponding protein	mutant phenotype	origin of the probe	genetic regulation	GenBank entry	reference
Major house-keeping genes											
1	*groEL*	*groEL*	2C4-2C7	nd	1	major chaperonin of the bacterial cell	essential for cell growth, assembly of enzymatic complexes**	sph	induced by heat shock	not in GenBank	R.Tabita, unpubl.
2	*dnaA, dnaN, gyrB*	*gyrB*	2G8-2G9	4 kb	3	DnaA, N - initiation of replication GyrB – β-subunit of topoisomerase II, responsible for negatively supercoiling DNA in an ATP-dependent manner	essential for cell growth maintaining supercoiling balance**	cap	regulated by changing negative supercoiling (indirect self-regulation)	not in GenBank	E. Sveen, unpubl (R.G. Kranz et al., 1992)
3	*parE*	*parE*	1A6	3.1 kb	1	subunit of topoisomerase IV, essential for cell partition, supposedly by resolving catenated chromosomes in *E. coli* and *Salmonella*	essential for cell growth (chromosome partition)**.	cap	nd	not in GenBank	E. Sveen, unpubl.
4	*nusG, rpl, rpoB, rpoC*	*rpoB*	2A3	7 kb	7	RpoB, C - β, β'-subunit of RNA polymerase NusG - antitermination *rpl* - ribosomal proteins (50S subunit)	essential for transcription**	cap	growth rate dependent	not in GenBank	B. Abella and L. Scappino, unpubl.
5-A9 6-B 7-C 8-D	*rrn* 4 clusters	S16' S23'	**A** 1D3, 4 **B** 1F6, 7 **C** 2A11, 2H5, 6; **D** 2G1, 3	1470 2884	8	ribosomal RNA	essential for protein synthesis (variations in number of intact copies are viable)**	cap sph	growth rate dependent	RCRN23S X06485 RCARRDA M34129	(A. Regensburger et al., 1988)
9	*recA*	*recA*	1D5, 1D6	1347	1	DNA binding protein and protease, key protein of homologous recombination and SOS-related functions	essential for recombination and DNA repair, very pleiotropic	sph	SOS inducible	RSRECA X72705	C. S. Garnica, unpubl.
10	*himA*	*ORF2*	1B1 (**795-1317**)	5403	3	α-subunit of IHF. Site specific (with consensus established for *E. coli*) DNA binding protein. Involved in pleotropic site-specific recombination and transcription regulation by DNA bending.	not essential for cell viability but may cause specific auxotrophies	cap	growth rate, SOS and self regulated	RCAHIMA M84030 M62764	(B. Toussaint et al., 1991)
11	*hip*	*hip*	1E10 (**16-476**)	479	1	β-subunit of IHF.	Same as for HimA.	cap	Same as HimA.	RCAHIPIHF L13998	(B. Toussaint et al., 1993)
12	*sigA*	*sigA*	2E11, 2E10	3.4 kb	3	major σ-subunit of RNA polymerase	essential gene	cap	nd	not in GenBank	S. Zheng, unpubl.

Table I. *Continued*

Genes involved in nitrogen fixation and linked with them											
13	*nif*-cluster A *(orf19, 18, 17, 16, 14, fdx P, N (C=P?), C, orf 9,10, nifENX-orf4-fdxB-orf5-nifQ-orf6-nifUSVW, nifA, nifB-orf1-nifZ)*	*nifA-2*	2F9-10	3570 4638 4282 2977 638 5540	15/ 26	Nitrogenase cofactor biosynthesis (major nitrogen fixation complex) and related proteins Orf18, 17 - Fe-S proteins FdxPN(C?) and B- ferrodoxins NifEN, Q and B - FeMoCo synthesis NifX- FeMoCo synthesis/regulation NifUS -iron cluster assembly NifV - homocitrate synthesis NifW - homocitrate processing NifA - nif specific transcriptional activator (final part of fixed N sensing cascade) NifZ - full activation or catalytic stability of FeMo protein of nitrogenase (nifDK)	*nifS*-Nif *nifV - leaky* Nif$^-$ *nifUI, UII, W* and *orf6* - Nif$^+$ *fdxN* and/or *fdxC* -Nif$^-$ *nifA, B* - Nif$^-$ (but in double mutant)	cap sph	nif regulatory cascade is induced by low oxygen pressure and low ratio glutamine/α-ketoglutorate	RCNIF X68444 RCNIFAB X07567 RCNIFS X17433115 RCAFDXC D1362523 RCAFDXN M31073 RCPTFAF1 X51316 RCANIFA M86823	(K. Saeki et al., 1990)
14	*rdxA*	*rdxA*	1E7-1E8 (**716-1256**)	3483	2	Homologous to *fixG*, the first gene of a *Rhizobium meliloti* nitrogen fixation operon on the pSym plasmid	not essential for nitrogen fixation in R. sphaeroides.	sph	nd	RCAFIXG M94725	(E.L. Neidle and S. Kaplan, 1992)
15	*nifHDK* cluster **B** *nifHDK, nifU-nifR4, nifA, nifB, fdxD*	*nifA-1* *nifR4*	1H3	2466 381 72 2423 1040 512 854 936 2583 2083	8	Nitrogenase and its regulation NifH - Fe protein of nitrogenase (major nitrogen fixation complex) containing [4Fe-4S] cluster NifDK - FeMo protein of nitrogenase, a tetramer containing 2 FeMo cofactors NifR4 - *nif* specific sigma factor FdxD -ferrodoxin	*nifH, D, K* and *R4* - Nif$^-$	cap	nif regulatory cascade	RCANIFHD M15270 RCANIFPRA M29400 RCANIFPRB M29401 RCFDXD X63352 RCNIFH X0786666. RCNIFK X63354 RCNIFKD X6335368. RCNIFKG X63196 RCNIFR4 X12358 RCNIFR4B X15437	(R. Jones and R. Haselkorn, 1989; W. Klipp et al., 1988; P. Preker et al., 1992)
16	*nifR1* cluster *niR1, nifR2, nifR3*	*nifR1*	1B7-9	8030	3	*nif* regulation homologous to NtrBC NifR2 - kinase phosphorylating NifR1 in response to changing concentration of GlnB-UMP	Nif$^-$	cap	nif regulatory cascade	RCNIFR12 X12359	(R. Jones and R. Haselkorn, 1989)
17	*adgA*	*adgA*	1D5,6	2432	1	ATP-binding protein involved in nitrogen metabolism	ammonia dependent growth in *R. capsulatus* (homolog is essential for growth of *E. coli)*	cap	nd	RCADGA X59399	(J.C. Willison, 1992)
18	*glnAB*	*glnA*	1C1, 2	1360 2460	2	*nif* regulation GlnB - target of uridylylation by UTase in response to low ratio of glutamine/α-ketoglutorate GlnA - glutamine synthetase	Tn*5* in *glnB* - constitutive *nif* expression, glutamine auxotrophy (polar effect) *glnA*- glutamine auxotrophy	cap sph	twofold induction by nitrogen limitation.(glnBA-lacZ translational fusion). NifR1 is responsible for 50% of the effect	RCAGLNAB M28244 RSBLNBAA X71659	(R.G. Kranz et al., 1990)
19	*glnQ*	*glnQ*	2A3	6.0 kb	4	glutamine permease	nd	cap	nd	not in GenBank	S. Zheng, unpubl.
20-A 21-B	*anfGDHA*	*anfGDHA*	**A**-2F3, 4 **B**-2C1, 2B12	nd	4	alternative nitrogenase	Nif$^-$ in absence of major Nif system	cap	repressed by Mo, NH_4 and O_2	not in GenBank	(K. Schuddekopf et al., 1993).

Table I. *Continued*

Genes of the photosynthetic apparatus											
22	*hemT*	*hemT*	1H3, 1H4	1881		Delta-aminolevulinic acid (common precusor of haem and protoporphyrin) synthase (ALAS) - isozyme of HemA	nd	sph	nd	RCAHEMTI L07489	(U. Hornberger et al., 1990) E.L. Neidle and S. Kaplan, 1993)
23	*reg* cluster (*ahcY hvrAB, regA, orf5 and 7*)	*regA- hvrA*	2H3, 2G11	1642 1105	4/6	regulation of the photosynthetic apparatus Ahc - adenosyl-homocysteine hydrolase. It controls ratio S-aden-met/S-aden-homocys that influences methylation of protoporphyrin in BChl biosynthesis RegA - anaerobic response activator of LH and RC gene expression hvr - nd	*ahc*$^-$ - methionine, homocysteine auxotrophy. *regA*$^-$ fails to transactivate LH and RC gene expression but not bch, produces ICM vesicles. Becomes essential in dim light	cap	*ahc* transcription is repressed by high light *regA* activity is regulated by putative sensor kinase via phosphorylation	RCAAHCY M80630 RCAREGA M64976	(M.W. Sganga et al., 1992)
24	*pucABCDE*	*pucA*	2C9	600 3169 743 2846 1078 437 2846	5	LH-II pucA, B - α and β polypeptides of the light-harvesting complex B800-850 (LH-II) pucC - essential for high level transcription of the genes of the LHII complex pucD, E (γ subunit of LH-II) - stabilize the B800-850 complex	LH-II$^-$	cap sph	anaerobic induction (30 fold)	RCALHII K0233734. RCAPUCOA M28510 RCAPUCAB M16777 RSPUCBAC X68796 RCALH2A M28360 RSB800AB X05200 RSPUCBAC X68796	(H.V. Tichy et al., 1989)
25	photosynthetic cluster	*puhA pufQ*	1G7-10	45959 4023 4844		biosynthesis of chlorophylls - *bch* genes; LH and RC proteins - *puh* and *puf* genes; biosynthesis of carotenoid - *crt* genes	Detailed description below		Detailed description below	RCPHSYNG Z11165 RCARC1 K01183 RCARC2 K01184 K01185 K01186	D. Burke, unpubl.
25.1	Bacterio chlorophyll biosynthetic genes. Three subclusters *bchB-M bchE-I bchC,A*	*puhA pufQ*	1G7-10	1194 314 760 5606	13	bchCA operon bch BFKHLM operon bchEJGDI operon different stages of the biosynthesis of bacteriochlorophyll	Unable to synthesize BChl or its precursors and grow photosynthetically, accumulate corresponding precursor	cap sph	Limited (3 times) anaerobic induction through complicated mechanism: weak moderately oxygen regulated and stronger oxygen dependent promotors (anaerobic induction), cotranscribed *puh* and *puf* genes have their own regulated promotors.	RCABCHC M29966 RCABCHH M34843. RCBCHC X16164 RSBCHPUF X68795 X63320	(C.L. Wellington and J.T. Beatty, 1989)
25.2	carotenoid cluster *crtA-K* and *crtJ*	*puhA pufQ*	1G7-10	1194 1660 1794	9	Nine *crt* genes were attributed to certain stages of C_{40} biosynthesis (carotenoids protect cells from photooxidative damage). Totally 13 steps of C_{40} biosynthesis were proposed	Unable to synthesize corresponding carotenoid, accumulate precursor , light sensitive	cap sph	Twofold anaerobic and high light intensity induction. (These results are interpreted based on the protective function of carotenoids under high light intensity in the presence of O_2).	RCABCHC M2996647. J04969 J04969. RSCRTD X63204	(G.A. Armstrong et al., 1989)
25.3	*puhA*	*puhA*	1G7-10	783 199 711	1	H polypeptide of the RC complex	LH$^-$	cap sph	The expression of the genes coding for RC-L, RC-M, and RC-H is coordinately regulated by light intensity and O_2 concentration (30 times induction). An increase in light intensity causes a decrease in	RSPUHAG X63378 RCAPUHA M14732 RCAPUFAB M15105	(Y.S. Zhu and J.E. Hearst, 1986)

Table I. *Continued*

25.4	*pufQBALM X*	*pufQ*	1G7-10	932 814 59 432 199 886	5/6	LH-II and RC complex PufBA- α and β subunits of LH-I; PufLM - L and M subunits of RC complex PufQ - required for BChl biosynthesis	LH$^-$	cap sph	The same as above	RCAPUFQ M2275236. RCAPUFQB M20141 J03183 S97551 S97551 RCAIMP J05098 RCAPUHA M14732 RCARCL M10206	(Y.S. Zhu and J.E. Hearst, 1986)
26	*ORF798*	*ORF798*	1A7	813	1	gene involved in oxygen-regulated expression of the *puf* and *puc* operons	nd	cap	nd	RCORF798A Z21973	M. Pollich, unpubl.
Carbohydrate biosynthesis and transport											
27	*cbbL (rbcL) crfX, fbpA, prkA, fbaA, rbcS**	*cbbL*	1H2, 1H3	5628* 98* 4453*	6*	large subunit of ribulose 1,5 bisphosphate carboxylase/oxygenase form I (RubisCO)	no phenotype due to suppression by the other form of RubisCO	sph	Light induction.	RCAFICO2F M64624 RCAPRKAA M28006. RCAPRKBA M68914	(J.L. Gibson et al., 1991)
28	*cbbM (rbpL) fbpB, prkB, tklB, gapB, fbaB**	*cbbM*	1B5, 1B6	2099*	6*	large subunit of ribulose 1,5 bisphosphate carboxylase/oxygenase form II (RubisCO)	no phenotype due to suppression by the other form of RubisCO	sph	Light induction.	RCAFBPPRK J02922	(J.L. Gibson et al., 1991)
29 30	*cbbX*	*cbbX*	**A**-2A5 **B**-1D1		1	ORF of unknown function, linked with *cbbS* in *R. sphaeroides*	nd	sph	nd	not in GenBank	R. Tabita, unpubl.
31	*ORF*	*ORF*	2F1, 2		1	gene required for photolithoautotrophic growth	nd	sph	nd	not in GenBank	R. Tabita, unpubl.
32	*cbbE*	*cbbE*	2A7			pentose-5-phosphate-3-epimerase	nd	sph		not in GenBank	R. Tabita, unpubl.
33	*fruB, K, A*	*fruA*	2C10, 11	5646 5642	3	Sugar transport and uptake FruA - integral membrane enzyme II (fructose permease) FruB - peripheral membrane enzyme I (multiphosphoryl transfer protein) FruK - soluble fructose-1-phosphate kinase	fruA::Tn*5* - fructose-negative, glucose-positive	cap	100-fold inducible (The uninduced mutant fruA exhibited measurable activities of both enzyme I and fructose-1-phosphate kinase, which were increased threefold when grown in the presence of fructose.)	RCAFRUAK M62785 M68879 X53150 RCFRUOP X53150	(L.F. Wu and M.J. Saier, 1990)
34	*dctS, dctR and dctP*	*dctP*	2E8, 9	2753 1062	3	DctP - major permease for the C4-dicarboxylates (malate, succinate and fumarate) aerobic dependent transport . (There is another anaerobic-light dependent low-affinity system). Dct S predicted to be a membrane bound sensor-kinase and DctR a response-regulator (all by comparison with FixJ)	*dctS* , *R* or *P* unable to grow on malate, succinate and fumarate in the dark due to the transport deficiency	cap	nd	RCDCTSRS X64733 RCDCTP X63974	(J.G. Shaw et al., 1991)

Table I. *Continued*

Genes coding electron transfer proteins											
35	*Nor?*	*Nor?*	1C9, 1C10 **(518-1018)**	1849	3	Subunits of the NADH:CoQ oxidoreductase must be clustered in the genome of R. capsulatus.	nd	sph		RCNDHAI Z11611	(A. Dupuis, 1992)
36	hydrogenase cluster *(hupR2-hyp-ORF20)*	*hupSL*	1G2-4	4175 2271 5456 4920 3052 4175 4674 4126 5456	18/20	HupSL - small and large subunits of hydrogenase. Its functions are H_2-uptake for authotrophic growth with H_2 as the electron source and aerobic oxidation of H_2 coupled with oxygen reduction. HypA, F, HupR1 - regulators HupD, G, C, D - hydrogenase processing HupM -e^- carrier HupJ - rubredoxin HypB - Ni incorporation HupF, H and K and HypE - unidentified but essensial	unable to consume H_2 (Hup^--phenotype)	cap	complicated pattern responding to H_2 (induction via HypF) and various environmental switches. Process involves HupR1 (response regulator activating expression in various conditions) and HypA (repressor of *hup*SL)	RCHUPLS X13520 RCHYPFG Z15087 RCHYPHUP X61007 RCORF1920 Z15088 RCAHYOX M55089 RCHUPR2U X57380 RCHUPXG Z15089 RCHYPHUP X61007	(A. Colbeau et al., 1993)
37	cytochrome c biogenesis cluster *ORF124, helABCDX, hpt, secDF*	*helAB*	1B9	760 3272 776	7/8	HelX - periplasmic disulphide oxidoreductase, involved in cytochrome c biogenesis. (c-type cytochromes have heme covalently attached to Cys in conserved motif (c_2 is periplasmic, c_1 is a component of the bc_1 complex) HelABCD-heme transporter in cytochrome c biogenesis SecDF - named by homology to the *E.coli* genes essential for protein export. Hpt - homolog of hypoxanthine phosphoribosyltransferase	*hel*$^-$ cannot grow anaerobically, unable to oxidize cytochrome specific electron donor TMPD It was not possible to inactivate secDF to see phenotype (presumably essential for cell growth).	cap	induced under dark growth anaerobic conditions	RCAHELDTRX M96013 RCHEL X63462 RCHPT X60977	(D.L. Beckman et al., 1992)
38	*ccl* claster *ccl1 ccl2 ORF257 argD*	*ccl1* and *2*	1F3-5	1002 3155	4	ORF257 - enoyl-CoA hydratase homologue (related to mitochondria but not to *E. coli* and peroxisomes) Ccl - required for the biogenesis of c-type cytochromes, demonstrate 52% homology with ORFs from chloroplasts	ccl$^-$ cannot grow anaerobically	cap	induced under dark growth anaerobic conditions	RC257 X60194 RCCCL12 X63461	(D.L. Beckman et al., 1992)
39	*cycA*	*cycA*	1E2-5	1242 1538 548	1	soluble periplasmic cytochrome c-2 performs electron transfer between membrane proton-translocating ubiquinol:cytochrome c_2 oxidoreductase (bc_1-complex) and cytochrome oxidase during respiration; and between bc_1 and oxidized photochemical RC during photosynthesis	not essential in *R.capsulatus* (function is duplicated by CytY), but essential in *R. sphaeroides*	cap sph	induced under dark anaerobic growth conditions	RCACYCA M12776 RCAC2AA M64777 RCACYC2 M14501	(F. Daldal et al., 1986)
40	*pet* cluster *petABC (fbcFBC)* and *perP, R*	*petABC*	1D8, 9	315 4007 1672 316 3381 3874	5	PetA - Rieske Fe-S protein PetB - cytochrome b PetC - cytochrome c_1 - ubiquinol-cytochrome c_2 oxidoreductase. Electron transfer in respiration and photosynthesis by oxidation of quinols and eventual reduction of soluble electron carrier. PetR deduced amino acid sequence is homologous to various bacterial response regulators, especially OmpR subgroup	PetABC are essential for photosynthetic growth. PetR is essential for photosynthetic and respiratory growth .	cap sph	nd	RCAPETAR M18576 RCPETG X05630 RCPETPR Z12113 RCAPETA M18577 RSFBCOPER X56157 RCFBC X03476	(M.K. Tokito and F. Daldal, 1992)

Table I. *Continued*

41	*cycI* and *adhI*	*cycI*	1F11	783		isocytochrome c2 structural gene	increase in the levels of CycI is necessary and sufficient for photosynthetic growth in the absence of Cyt c2	sph	nd	RCACYCI L02104	(M.A. Rott et al., 1993)
42	*fbcQ*	*fbcQ*	1C1	645	1	integral part of b-c_1 complex of *R. sphaeroides*	nd	sph	nd	RCAFCBQ M68939	(S. Usui and L. Yu, 1991)
43	*cyt* c_y	*cyt* c_y	2C8 **(425-960)**	1190 b	2	electron transfer between membrane proton-translocating ubiquinol:cytochrome c_2 oxidoreductase (bc_1-complex) and cytochrome oxidase during respiration; and between bc1 and oxidized photochemical RC during photosynthesis. Able to replace C_2 (located in periplasm)	complement *R. speroides cyt* c_2 to PS^+ phenotype Double mutant of *R. capsulatus* is unable to grow photosynthetically	cap	nd	RCCYCYA Z21797	(F.J. Jenney and F. Daldal, 1993)
44	*trxA*	*trxA*	1B1, 1A12 **(14-454)**		1	thioredoxin	nd	sph	nd	RCATRXA M33806	(S. Pille et al., 1990).
Other metabolic genes											
45	*mtlK*	*mtlK*	1F5 **(1553-1058)**	2kb	1	mannitol dehydrogenase	loss of the ability to grow on D-mannitol	sph	nd	RCAMTLKA	(K.H. Schneider et al., 1993)
46	*cpeA*	*cpeA*	1B10, 1B11 **(402-902)**	1731	1	catalase-peroxidase	nd	cap	regulated by oxygen		(H. Forkl et al., 1993)
47	sucA	sucA	1G5	332 360	1	α-ketoglutarate dehydrogenase	nd	cap	nd	RCASUCAA L10207 RCASUCAB L10208	F. Dastoor, upubl.
48	trpC	trpC	2B9-11	801	1	indolglycerol phosphate synthase	Tp^{-**}	cap	repression, attenuation, rel control system**	RCATRPC M97640	(R.M. Becker et al., 1992)

cap -*R. capsulatus*
sph - *R. sphaeroides*
*- in *R.sphaeroides*
#-coordinates of the amplified fragment used as a probe for mapping (first np is the beginning of the GenBank entry)
** - in *E. coli*
nd - not described
To limit the number of references, we usually provide one per gene mapped. GenBank accession numbers listed in the table can be used as a source of additional references.

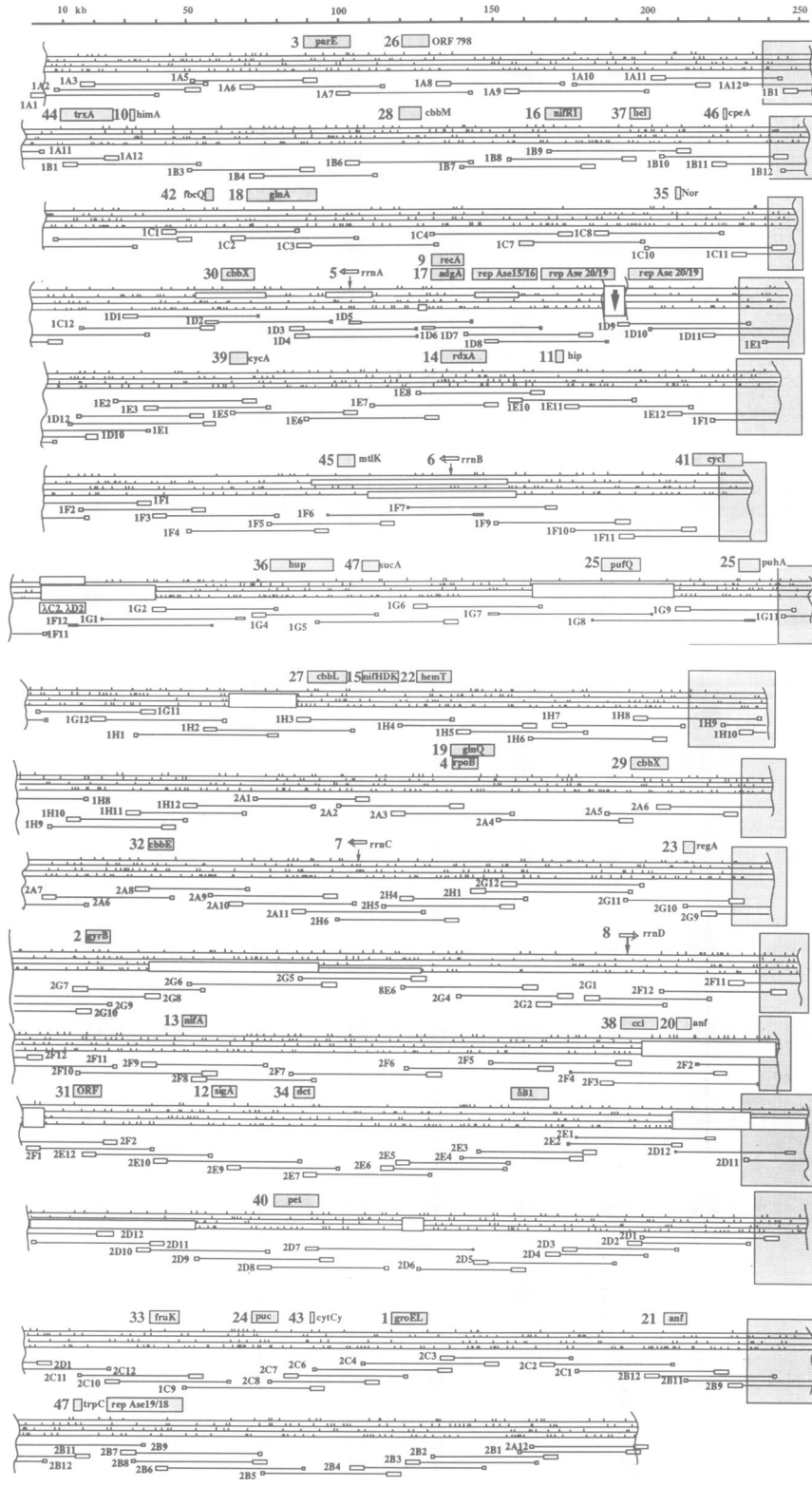

Fig. 2. Combined high-resolution physical and genetic map of the chromosome of *R.capsulatus* SB1003: Four horizontal lines with vertical ticks for restriction sites represent the physical map of the *Rhodobacter* chromosome (*Eco*RV, *Bam*HI, *Hin*dIII and *Eco*RI from top to bottom) generated by cosmid merging. Sites mapped only by terminase are represented by faint vertical ticks. Areas where maps are unavailable are covered by empty rectangles. One gap is marked by the black arrow in a rectangle. An unmapped λ clone covering the other gap is represented by a rectangle with the names of λ clones in it. Each cosmid is drawn as a horizontal line under the map. The name of each cosmid is on its left, e.g. 1A1, 1A2, etc. On each cosmid, the L and R cos sites are shown by large and small boxes, respectively. Mapped genes and repeated elements are indicated by boxes above the map with numbers corresponding to their numbers in Table I. δB1 is deleted in the B1 strain. The size of the rectangle corresponds to the minimal hybridizing element of the map. The physical map is read continuously from left to right. Ten to twenty kb marked by the gray background at the end of each 250 kb stretch of the map is repeated at the beginning of the next piece to provide visual continuity. The scale in kb is above the map.

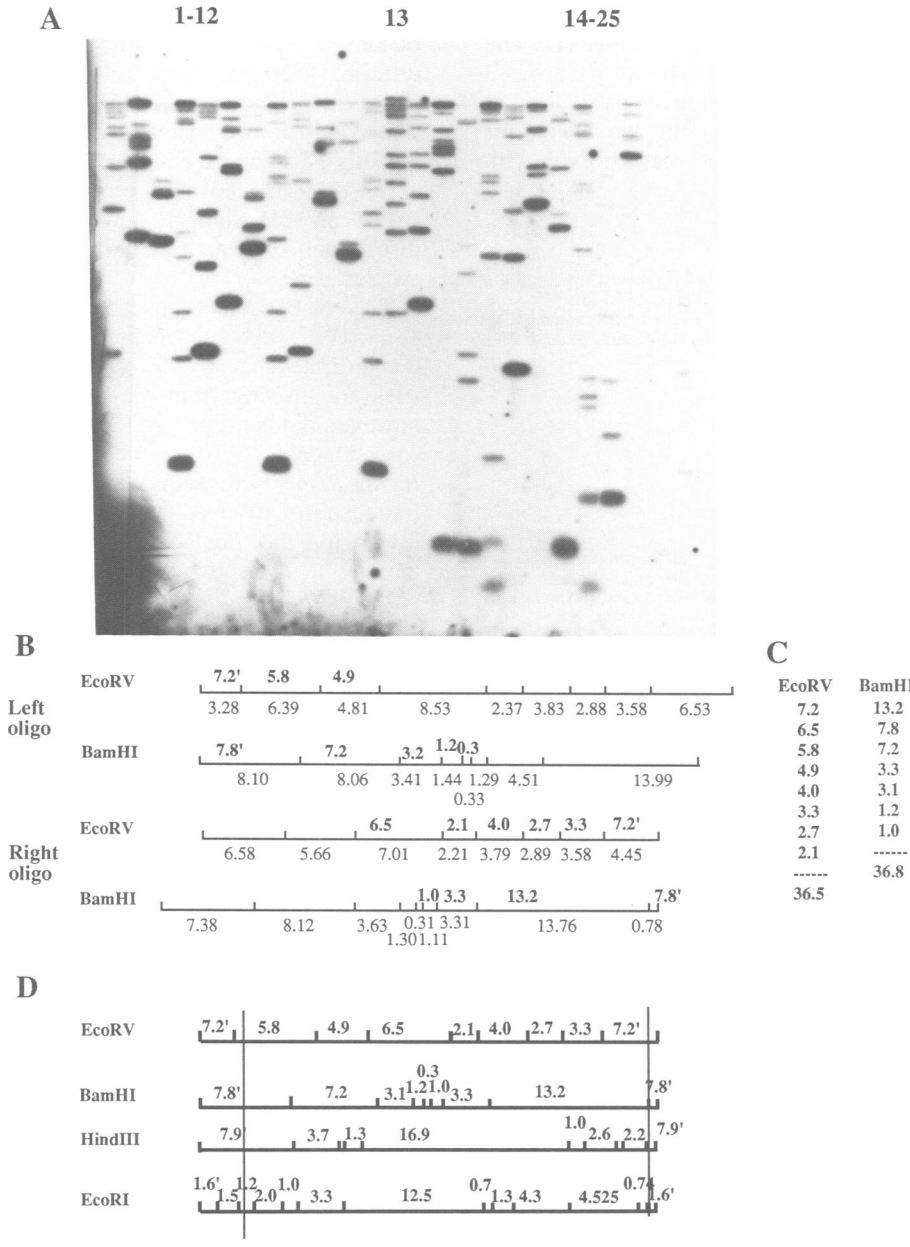

Fig. 3. Physical mapping of cosmids by partial digestion with *Eco*RV, *Bam*HI, *Hin*dIII and *Eco*RI and *cos*-site end-labeling: (**A**) Autoradiogram: lane 13, Takara DNA marker (4.6–48.5 kb); lanes 1–12, digested cosmid DNA annealed with *cosL*; lanes 14–24, digested cosmid DNA annealed with *cosR*. (**B**) 'Rough' data generated by the GelSize program, with data from complete digests incorporated (in bold). (**C**) Fragment sizes calculated from complete digests. (**D**) Combined final map of cosmid 2E2.

Only heat-shock-induced RNA produced a hybridization pattern distinct from 'standard conditions' RNA. About a dozen bands were found 3–10 times more intense than the control (Figure 6D—only half of the HRHT shown). The intensity of a given band should be compared with the intensity of the surrounding unchanged bands. Heat shock also repressed ~20 areas strongly expressed in standard conditions.

Discussion

A high-resolution physical map of the chromosome of *R.capsulatus* SB1003 was assembled into one contig consisting of two stretches of cloned DNA fragments. These two stretches are linked by the 7/8-*Ase*I–*Xba*I fragment (Fonstein *et al.*, 1992) covering a 1–10 kb gap, most likely due to the unclonability of a certain genetic element. Such genes have been found in many organisms (Birkenbihl and Vielmetter, 1989; Bukanov and Berg, 1994) and are known to be common obstacles in the construction of gene encyclopedias. The nature of this unclonable element can be further studied by PCR amplification of the DNA fragment lacking from the cosmid library and subsequent sequencing. Another gap in the map, mistakenly linked earlier (Fonstein and Haselkorn, 1993), is thought to be due to telomere-type structures recently found in *Streptomyces* species (Leblond *et al.*, 1993; Lin *et al.*, 1993). The initial linkage of

macrorestriction fragments that circularized the long-range physical map of *R.capsulatus* between cosmids 1D8 and 1D9 (Fonstein *et al.*, 1992) turned out to be due to repeated DNA in these cosmids. Cross-hybridizing structures at or near the ends of these cosmids may be responsible for this phenomenon, but further study is needed. Even with these gaps, the genome of *R.capsulatus* SB1003 consists of one 3.7 Mb chromosome and a 130 kb plasmid, differing from its close relative *R.sphaeroides*, which carries two chromosomes (Suwanto and Kaplan, 1989). The alignment of a cosmid encyclopedia of *R.sphaeroides*, recently generated for its small chromosome (Choudhary *et al.*, 1994), along the physical map of *R.capsulatus* would provide valuable information on continuity and distribution of genetic material for these closely related species.

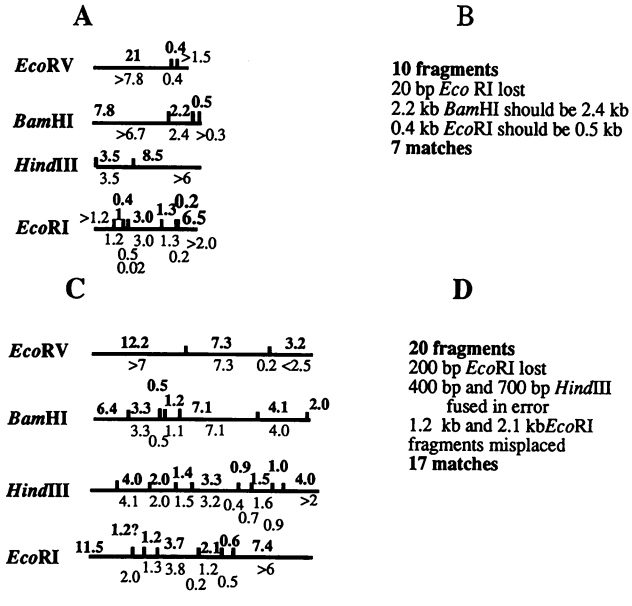

Fig. 4. Comparison of mapping results with data derived from sequencing. (**A**) 9 kb region of *hup* gene cluster compared with part of cosmid 1G3 (experimental data are in bold); (**C**) 17 kb of the photosynthetic gene cluster assembled from three GenBank entries compared with part of cosmid 1G9; (**B**) and (**D**) summaries of this comparison.

The comparison of the restriction map of *E.coli* (Kohara *et al.*, 1987) with sequencing data (Churchill *et al.*, 1990) demonstrated that up to 5% of the closely spaced restriction sites are missing from the physical map. This finding, however, did not diminish the usefulness of this map. Our comparison of a 30 kb piece of the restriction map of *R.capsulatus* with sequencing data revealed the same frequency of discrepancies. The accuracy in our data is lower for the *Eco*RI sites, where the mean size of the fragments is two times smaller, than for the other enzymes used.

Chromosomal replication coincides with the direction of transcription of the *rrn* operons in all microorganisms studied. According to this principle, the replication Ori should be located between the two closest *rrn* operons that are transcribed in different directions, namely, on a 350 kb fragment between the *rrnC* and *rrnD* operons.

The use of HRHT for surveying total RNA pools was demonstrated. It is possible to locate all genetic elements related to a process studied in one experiment, if the level of induction of their transcription exceeds 50%. However, this factor can be masked by the large size of many of the *Eco*RV fragments on the map. With the current

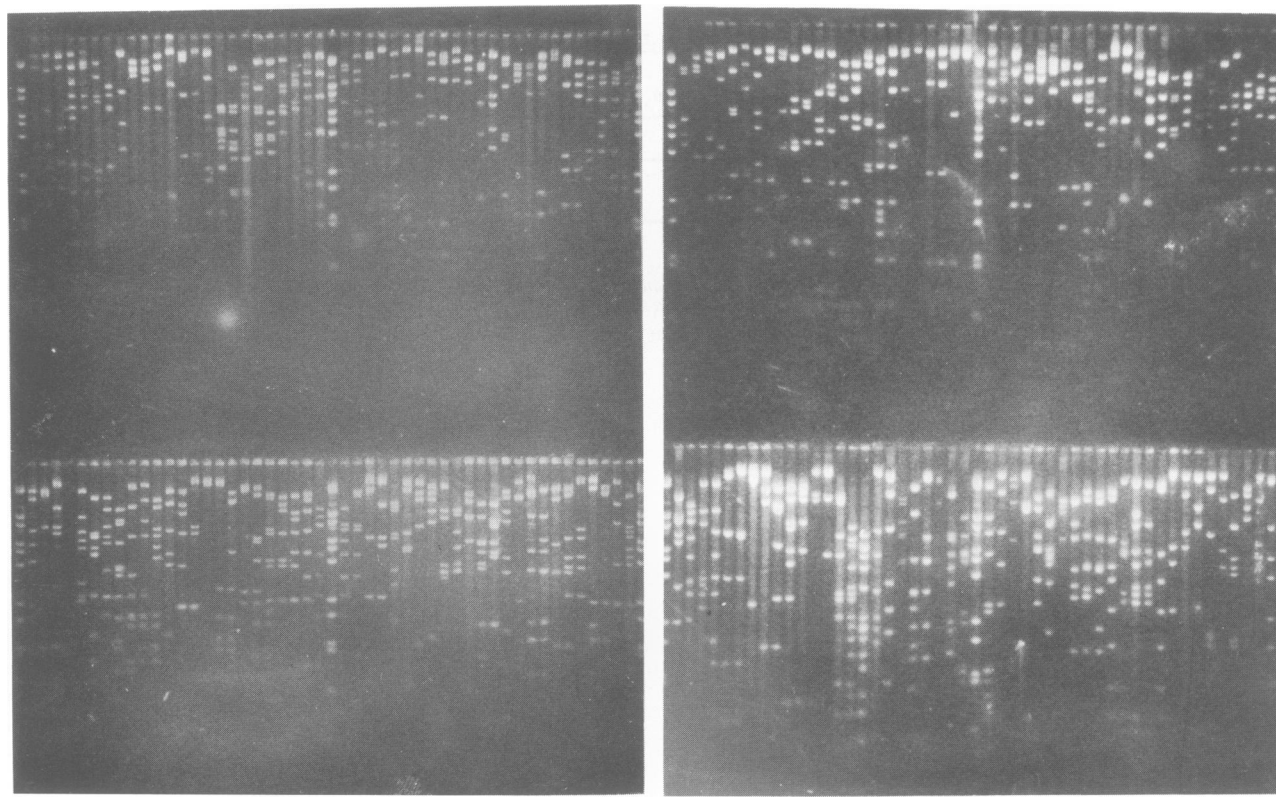

Fig. 5. *Eco*RV restriction patterns of all 192 cosmids from the minimal set, comprising the entire *Rhodobacter* genome.

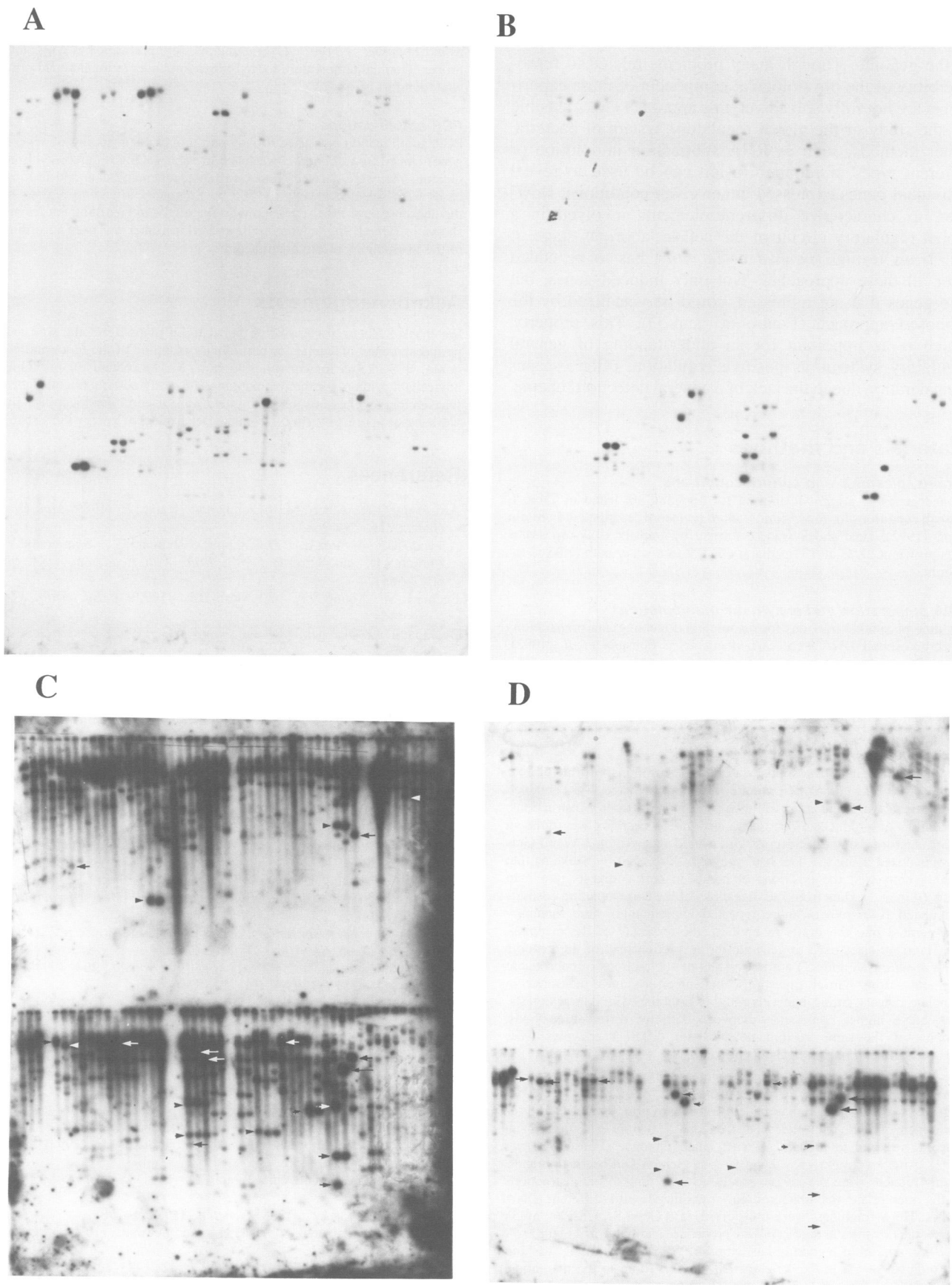

Fig. 6. Probing of the HRHT. (**A**) Cosmids 1–96 probed with the *P* gene of a permease operon. (**B**) Cosmids 1–96 probed with the *Q* gene of the same permease operon. (**C**) Cosmids 1–96 probed with RNA generated under 'standard conditions'. (**D**) Cosmids 1–96 probed with RNA generated under 'heat shock' conditions: arrows pointed leftwards mark induced genome regions, arrows pointed rightwards mark repressed ones.

improvement of the resolution of the map, it is possible to produce an HRHT with almost gene-size fragmentation of the genome. Though many problems related to reproducibility of the physiological component of these experiments are not fully solved yet, this approach looks feasible for the study of the global regulatory system of bacteria. Other methods, such as RNA subtraction in solution or different types of reporter fusion can be used to reveal individual genes expressed under given conditions. However, to characterize different elements involved in a global regulatory circuit of the cell, a laborious stage of *cis–trans* testing (*in vivo* or *in vitro*) has to be added after all these approaches. Not only induced genes, but also genes that are repressed, could be visualized by the proposed approach (Figure 6C and D). This property, which is as important for an understanding of general regulatory systems as positive regulation, often escapes consideration due to the lack of a general detection scheme.

Materials and methods

Strains, plasmids and culture conditions

Cloned genes of *Rhodobacter* used for mapping are listed in Table I. Growth conditions for *R.capsulatus* and *E.coli* were described in Fonstein *et al.* (1992). Heat shock was performed by transfer of *R.capsulatus* cells grown at 28°C in YT media at OD 0.2 to a water bath at 45°C for 10 min.

DNA preparation and enzymatic manipulations

Alkaline plasmid extractions for mapping and labeling and preparations of chromosomal DNA were carried out as in Fonstein *et al.* (1992). Restriction endonucleases and Klenow fragment of DNA polymerase were purchased from New England BioLabs and used according to their instructions. Enzymes for SP6 or T7 *in vitro* transcription were obtained from Promega Corp and used according to their manual.

Terminase mapping

Terminase mapping of cosmids was done according the principles of Rackwitz *et al.* (1985). Terminase for *cos* cleavage was obtained from Takara Corp and used following the manufacturer's instructions. Separation of products of partial digestions of cosmids was done in 0.35% agarose using 25 cm gels with a field strength of 3 V/cm. Gels were analyzed using the 'GelSize' program developed by Buikema that directly converted the positions of bands in partial digests into map drafts (Figure 3). Data from full digestions of the corresponding cosmids (calculated from two or more repeated experiments) were manually incorporated into the drafts to increase the accuracy of the mapping. The fragment sizes used to generate the restriction map of the genome of *R.capsulatus* (Figure 2) are not results of subtractions of consequent fragments from partial digestions, but precise values measured in complete digests, fitting intervals roughly established in partial digests. To achieve higher consistency between data for different enzymes, samples of the same cosmid treated with different enzymes were separated in neighboring lanes of the gel.

Hybridization analysis of cosmid library

A device was constructed that made it possible to print 96 *E.coli* cultures from each of nine microtiter plates on one 150 mm Petri dish in an ordered way and to prepare six such plates at once (Fonstein *et al.*, 1992). After overnight growth on Colony Screen DuPont membranes, colony replicas were picked up, processed according to the instructions of the membrane manufacturer and hybridized with different DNA probes. DNA fragments were transferred onto Gene Screen Plus nylon membranes by the standard capillary procedure and hybridized with 0.5–2×10^6 c.p.m./ml of randomly labeled probe. The hybridization, washing and removal of the probe were carried out according to the membrane manufacturer's protocol. The only exception was the final 0.1×SSC wash, performed at 65–75°C. Filters were exposed to Kodak X-ray film for between 2 and 48 h. Whilst being kept wet, each filter was used for between five and seven hybridizations, removing the previous probe by incubation in 0.2 N NaOH for 40 min at 42°C.

RNA manipulations

Total RNA samples were extracted and labeled by reverse transcriptase as in Chuang *et al.* (1993). Hybridizations were performed as with other probes. Optimal temperature for hybridization under stringent conditions was found to be 68–72°C.

PCR amplifications

PCR amplification was used to generate probes for mapping of several genes cloned from *Rhodobacter*, listed in Table I. Oligos purchased from Operon, Inc. were derived from GenBank sequencing data and in most cases were selected to amplify 500 bp fragments in coding regions of the chosen genes. Precise positions of the oligos in GenBank entries are shown in Table I. *Taq* polymerase from Perkin-Elmer was used according to the instructions of the manufacturer.

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References

Armstrong,G.A., Alberti,M., Leach,F. and Hearst,J.E. (1989) *Mol. Gen. Genet.*, **216**, 254–268.
Azevedo,V., Alvarez,E., Zumstein,E., Damiani,G., Sgaramella,V., Ehrlich,S.D. and Serror,P. (1993) *Proc. Natl Acad. Sci. USA*, **90**, 6047–6051.
Becker,R.M., Young,D.A. and Marrs,B.L. (1992) *J. Bacteriol.*, **174**, 5482–5484.
Beckman,D.L., Trawick,D.R. and Kranz,R.G. (1992) *Genes Dev.*, **6**, 268–283.
Birkenbihl,R.P. and Vielmetter,W. (1989) *Nucleic Acids Res.*, **17**, 5057–5069.
Bukanov,N.O. and Berg,D.E. (1994) *Mol. Microbiol.*, **11**, 509–523.
Charlebois,R.L., Schalkwyk,L.C., Hofman,J.D. and Doolittle,W.F. (1991) *J. Mol. Biol.*, **222**, 509–524.
Choudhary,M., Mackenzie,C., Nereng,K., Sodergren,E., Weinstock,G. and Kaplan,S. (1994) In *Proceedings of the VIII International Symposium on Phototrophic Procaryotes.* Urbino, p. 114.
Chuang,S.E., Daniels,D.L. and Blattner,F.R. (1993) *J. Bacteriol.*, **175**, 2026–2036.
Churchill,G.A., Daniels,D.L. and Waterman,M.S. (1990) *Nucleic Acids Res.*, **18**, 589–597.
Colbeau,A., Richaud,P., Toussaint,B., Caballero,J., Elster,C., Delphin,C., Smith,R.L., Chabert,J. and Vignais,P.M. (1993) *Mol. Microbiol.*, **8**, 15–29.
Daldal,F., Cheng,S., Applebaum,J., Davidson,E. and Prince,R.C. (1986) *Proc. Natl Acad. Sci. USA*, **83**, 2012–2016.
Donohue,T.J. and Kaplan,S. (1991) *Methods Enzymol.*, **204**, 459–485.
Dupuis,A. (1992) *FEBS Lett.*, **301**, 215–218.
Eiglmeier,K., Honore,N., Woods,S.A., Caudron,B. and Cole,S.T. (1993) *Mol. Microbiol.*, **7**, 197–206.
Fonstein,M. and Haselkorn,R. (1993) *Proc. Natl Acad. Sci. USA*, **90**, 2522–2526.
Fonstein,M., Zheng,S. and Haselkorn,R. (1992) *J. Bacteriol.*, **174**, 4070–4077.
Fonstein,M., Nikolskaya,T., Zaporojets,D., Nikolsky,Y., Kulakauskas,S. and Mironov,A. (1994) *J. Bacteriol.*, **176**, 2265–2271.
Forkl,H., Vandekerckhove,J., Drews,G. and Tadros,M.H. (1993) *Eur. J. Biochem.*, **214**, 251–258.
Gibson,J.L., Falcone,D.L. and Tabita,F.R. (1991) *J. Biol. Chem.*, **266**, 14646–14653.
Hill,C.W. and Gray,J.A. (1988) *Genetics*, **119**, 771–778.
Hornberger,U., Liebetanz,R., Tichy,H.V. and Drews,G. (1990) *Mol. Gen. Genet.*, **221**, 371–378.
Jenney,F.J. and Daldal,F. (1993) *EMBO J.*, **12**, 1283–1292.
Johnson,J.A., Wong,W.K. and Beatty,J.T. (1986) *J. Bacteriol.*, **167**, 604–610.
Jones,R. and Haselkorn,R. (1989) *Mol. Gen. Genet.*, **215**, 507–516.
Klipp,W., Masepohl,B. and Puhler,A. (1988) *J. Bacteriol.*, **170**, 693–699.
Kohara,Y., Akiyama,K. and Isono,K. (1987) *Cell*, **50**, 495–508.

Kranz,R.G., Pace,V.M. and Caldicott,I.M. (1990) *J. Bacteriol.*, **172**, 53–62.
Kranz,R.G., Beckman,D.L. and Foster,H.D. (1992) *FEMS Microbiol. Lett.*, **72**, 25–32.
Kuspa,A., Vollrath,D., Cheng,Y. and Kaiser,D. (1989) *Proc. Natl Acad. Sci. USA*, **86**, 8917–8921.
Leblond,P., Redenbach,M. and Cullum,J. (1993) *J. Bacteriol.*, **175**, 3422–3429.
Lin,Y.-S., Kieser,H.M., Hopwood,D.A. and Chen,C.W. (1993) *Mol. Microbiol.*, **10**, 923–933.
Liu,S.L., Hessel,A. and Sanderson,K.E. (1993) *Proc. Natl Acad. Sci. USA*, **90**, 6874–6878.
Marrs,B. (1981) *J. Bacteriol.*, **146**, 1003–1012.
Neidle,E.L. and Kaplan,S. (1992) *J. Bacteriol.*, **174**, 6444–6454.
Neidle,E.L. and Kaplan,S. (1993) *J. Bacteriol.*, **175**, 2304–2313.
Pille,S., Chuat,J.C., Breton,A.M., Clement,M.J. and Galibert,F. (1990) *J. Bacteriol.*, **172**, 1556–1561.
Preker,P., Hubner,P., Schmehl,M., Klipp,W. and Bickle,T.A. (1992) *Mol. Microbiol.*, **6**, 1035–1047.
Rackwitz,H.R., Zehetner,G., Murialdo,H., Delius,H., Chai,J.H., Poustka,A., Frischauf,A. and Lehrach,H. (1985) *Gene*, **40**, 259–266.
Regensburger,A., Ludwig,W., Frank,R., Blocker,H. and Schleifer,K.H. (1988) *Nucleic Acids Res.*, **16**, 2343.
Riley,M. (1993) *Microbiol. Rev.*, **57**, 862–952.
Rott,M.A., Witthuhn,V.C., Schilke,B.A., Soranno,M., Ali,A. and Donohue,T.J. (1993) *J. Bacteriol.*, **175**, 358–366.
Saeki,K., Miyatake,Y., Young,D.A., Marrs,B.L. and Matsubara,H. (1990) *Nucleic Acids Res.*, **18**, 1060.
Schneider,K.H., Giffhorn,F. and Kaplan,S. (1993) *J. Gen. Microbiol.*
Schuddekopf,K., Hennecke,S., Liese,U., Kutsche,M. and Klipp,W. (1993) *Mol. Microbiol.*, **8**, 673–684.
Sganga,M.W., Aksamit,R.R., Cantoni,G.L. and Bauer,C.E. (1992) *Proc. Natl Acad. Sci. USA*, **89**, 6328–6332.
Shaw,J.G., Hamblin,M.J. and Kelly,D.J. (1991) *Mol. Microbiol.*, **5**, 3055–3062.
Smith,C.L., Econome,J.G., Schutt,A., Kleo,A. and Cantor,C.R. (1987) *Science*, **236**, 1448–1453.
Suwanto,A. and Kaplan,S. (1989) *J. Bacteriol.*, **171**, 5850–5859.
Tichy,H.V., Oberle,B., Stiehle,H., Schiltz,E. and Drews,G. (1989) *J. Bacteriol.*, **171**, 4914–4922.
Tokito,M.K. and Daldal,F. (1992) *Mol. Microbiol.*, **6**, 1645–1654.
Toussaint,B., Bosc,C., Richaud,P., Colbeau,A. and Vignais,P.M. (1991) *Proc. Natl Acad. Sci.USA*, **88**, 10749–10753.
Toussaint,B., Delic,A.I., De,Sury, D'Aspremont,R., David,L., Vincon,M. and Vignais,P.M. (1993) *J. Bacteriol.*, **175**, 6499–6504.
Trieselmann,B.A. and Charlebois,R.L. (1992) *J. Bacteriol.*, **174**, 30–34.
Usui,S. and Yu,L. (1991) *J. Biol. Chem.*, **266**, 15644–15649.
Wellington,C.L. and Beatty,J.T. (1989) *Gene*, **83**, 251–261.
Wenzel,R. and Herrmann,R. (1989) *Nucleic Acids Res.*, **17**, 7029–7043.
Willison,J.C. (1992) *J. Bacteriol.*, **174**, 5765–5766.
Willison,J.C. (1993) *FEMS Microbiol. Rev.*, **10**, 1–38.
Willison,J.C., Ahombo,G., Chabert,J., Magnin,J.P. and Vignais,P.M. (1985) *J. Gen. Microbiol.*, **131**, 3001–3015.
Wu,L.F. and Saier,M.J. (1990) *J. Bacteriol.*, **172**, 7167–7178.
Yen,H.C. and Marrs,B.L. (1976) *J. Bacteriol.*, **126**, 619–629.
Yen,H.C., Hu,N.T. and Marrs,B.L. (1979) *J. Mol. Biol.*, **131**, 157–168.
Zhu,Y.S. and Hearst,J.E. (1986) *Proc. Natl Acad. Sci. USA*, **83**, 7613–7617.

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