Genetic analysis of a bacterial genetic exchange element: The gene transfer agent of *Rhodobacter capsulatus*

Andrew S. Lang and J. T. Beatty*

Department of Microbiology and Immunology, University of British Columbia, 6174 University Boulevard, Vancouver, British Columbia, Canada, V6T 1Z3

An unusual system of genetic exchange exists in the purple nonsulfur bacterium *Rhodobacter capsulatus*. DNA transmission is mediated by a small bacteriophage-like particle called the gene transfer agent (GTA) that transfers random 4.5-kb segments of the producing cell's genome to recipient cells, where allelic replacement occurs. This paper presents the results of gene cloning, analysis, and mutagenesis experiments that show that GTA resembles a defective prophage related to bacteriophages from diverse genera of bacteria, which has been adopted by *R. capsulatus* for genetic exchange. A pair of cellular proteins, CckA and CtrA, appear to constitute part of a sensor kinase/response regulator signaling pathway that is required for expression of GTA structural genes. This signaling pathway controls growth-phase-dependent regulation of GTA gene messages, yielding maximal gene expression in the stationary phase. We suggest that GTA is an ancient prophage remnant that has evolved in concert with the bacterial genome, resulting in a genetic exchange process controlled by the bacterial cell.

Bacteria exchange DNA by three mechanisms: natural transformation, conjugation, and transduction. Transformation occurs when naked DNA is taken up from the environment. Conjugation results in transfer of plasmid or transposon sequences and possibly genomic sequences if a mobile genetic element has integrated into the chromosome. Transduction occurs when bacterial DNA is abnormally encapsidated in a bacteriophage (phage) particle and subsequently injected into another cell. Incoming DNA may either be degraded and the nucleotides recycled, replicate autonomously of the chromosome, or integrate into the chromosome (1).

A genetic exchange process that resembles transduction and is dependent on a phage-like particle was discovered in the bacterium *Rhodobacter capsulatus*. Because of its ability to transfer genes between *R. capsulatus* cells, this genetic element was given the name "gene transfer agent" (GTA) (2). GTA particles contain an apparently random distribution of 4.5-kb linear, double-stranded DNA fragments of the *R. capsulatus* genome (including plasmids) (3–5). Structurally, GTA resembles a small, tailed phage (4), but it does not possess any of the functions, such as plaque formation and transmission of viral genes, that are typically associated with phages (4, 6). Therefore, the sole activity of GTA is in gene transfer. Strains of *R. capsulatus* isolated from varied geographic locations produce GTA (2, 7), suggesting that GTA is an evolutionarily old phenomenon retained amongst most extant strains. Other phage-like agents of genomic gene transfer have been subsequently discovered in several other bacteria (8–10).

The unusual properties of GTA led to the suggestion that it might represent an evolutionary precursor of a phage or, alternatively, a defective phage (4). This paper reports the cloning and analysis of GTA structural and regulatory genes to attempt to distinguish between these two possibilities.

Materials and Methods

Growth Conditions of Bacterial Strains. The *Escherichia coli* strains used for cloning and subcloning were DH5α (GIBCO/BRL), DH10B (GIBCO/BRL), and the *dam* mutant RB404 (11). Strains S17–1 (12), C600(pDPT51) (13), and HB101(pRK2013) (14) were used to conjugate plasmids into *R. capsulatus*. *E. coli* strains were grown in Luria–Bertani medium (15) supplemented with the appropriate antibiotics at the following concentrations: ampicillin, 200 μg/ml; tetracycline-HCl, 10 μg/ml; kanamycin sulfate 50 μg/ml; and gentamycin sulfate, 8 μg/ml.

R. capsulatus strain Y262 (4) was used as the starting strain for subsequent strain construction (see below), and strain B10 (2) was used as the source of DNA for sequencing. Other DNA sequences used for analysis were from the closely related strain SB1003 (16). Strain SB1003 was constructed from a clone of strain B10 by GTA-mediated transfer of the rifampicin resistance marker from strain BB101 (3). Strain Y262 is a GTA-overproducer strain obtained by mutagenesis of strain BB103, a spontaneous streptomycin-resistant derivative of strain B10 (17). Strain BY1653 (18) was the indicator strain in the overlay screening, and the *puhA* deletion mutant strain DW5 (19) was used as the recipient for GTA assays. *R. capsulatus* strains were grown aerobically in RCV medium (20) supplemented with the appropriate antibiotics at the following concentrations: tetracycline-HCl, 0.5 μg/ml; kanamycin sulfate, 10 μg/ml; and gentamycin sulfate, 3 μg/ml; or grown photosynthetically in yeast extract/peptone/salts (YPS) medium (7).

Protein Sequencing. GTA was purified as described (4), and proteins were separated by SDS-PAGE and blotted onto a poly(vinylidene difluoride) membrane (Schleicher & Schuell). The N-terminal sequence of a protein band of ≈30 kDa was obtained by the University of British Columbia Nucleic Acid and Protein Service Unit (Vancouver, Canada).

Construction and Isolation of Mutants. A Tn5 mutant library of ≈2,000 members was constructed from *R. capsulatus* strain Y262 by using *E. coli* S17–1(pSUP2021) (12). Potential mutants were screened for loss of GTA production by using a multi-layered plate method previously described (4) with strain BY1653 as the indicator strain. Candidates were further evaluated for GTA production as described previously (6) with strain DW5 as the recipient and selection for photosynthetic growth. Gene disruptions were made using the *neo* gene-containing *Sma*I fragment (KIXX cartridge) from the plasmid pUC4KIXX (21) and subsequently transferred to the *R. capsulatus* chromosome by GTA transduction as described (5).
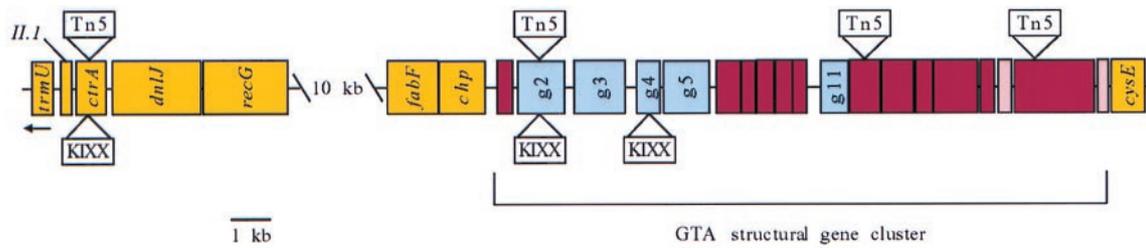
MICROBIOLOGY

Fig. 1. Map of a region of the *R. capsulatus* chromosome containing genes necessary for GTA production. Locations of Tn5 and KIXX cartridge insertion found to abolish GTA production are shown. All ORFs are predicted to be transcribed left to right except for *trmU*. ORFs with weak or no similarity to known genes are shown in red or pink; ORFs homologous to known phage genes are shown in light blue; ORFs highly homologous to known cellular genes are shown in yellow. All predicted ORFs match *R. capsulatus* codon usage well, as defined by the highly expressed photosynthesis genes, except for those shown in pink (orfg17 and orfg19). More detailed information about sequence similarities is given in Table 1. Information about the sources of the DNA sequences used to construct this figure is in the *Results* section.

Strain YCKF was constructed from strain Y262 by deleting the *ctrA*-coding region between nucleotides 124 and 672 and inserting a transcriptionally congruent KIXX cartridge in this site.

Alignments and Database Homology Searches. DNA and protein sequence alignments were done with the program GENEWORKS (Oxford Molecular, Campbell, CA). Database searches were performed with the predicted amino acid sequences of ORFs using the BLAST program (22).

DNA Manipulations and Plasmid Construction. Standard methods of DNA purification, restriction enzyme digestion, and other modification techniques were used (15). The plasmid pCTRA was constructed by inserting the strain B10 *ctrA* gene 3′ of the *puf* promoter in plasmid pRR5C (23) as an *Eco*RI–*Sma*I fragment. The plasmid pSTU12 was constructed by inserting an orfg2 *Pst*I–*Hin*dIII fragment from strain B10 (made blunt at the *Hin*dIII end with mung bean nuclease) into the plasmid pXCA601 (24), which had been digested with *Pst*I and *Bam*HI (with the *Bam*HI end made blunt with the Klenow enzyme). This resulted in an in-frame translational fusion between orfg2′ and ′*lacZ* under the control of the GTA gene promoter.

A *Xho*I–*Hin*dIII fragment containing the GTA promoter region from the Y262 chromosome was used as the source of 5′ sequences for construction of the orfg2′::′*lacZ* fusions used in β-galactosidase assays. A *Xho*I–*Sal*I fragment, made blunt with the Klenow enzyme, was ligated into the *Sma*I site of pBluescript I KS (Stratagene), and a *Pst*I–*Bam*HI fragment was removed from the resultant plasmid and inserted into the promoter probe vector pXCA601 to generate pYP, which contains a translationally in-frame fusion between orfg2′ and ′*lacZ* under the control of the GTA promoter. An analogous procedure was used to generate pYNP, which lacks the GTA promoter: a Klenow-blunted *Eco*RI–*Sal*I fragment was ligated into the *Sma*I site of pBluescript I KS and a *Pst*I–*Bam*HI fragment from the resultant plasmid subcloned into pXCA601, again producing an orfg2′::′*lacZ* translational fusion.

β-Galactosidase Assays. Photosynthetically grown *R. capsulatus* cells were harvested at the desired point in the growth phase, as determined by culture turbidity, and β-galactosidase activities were determined as described (25) on duplicate samples.

RNA Isolation and Probe Construction. Photosynthetically grown *R. capsulatus* cells were harvested at the desired point in the growth phase, as determined by culture turbidity, and RNA was isolated using the RNeasy kit (Qiagen). RNA electrophoresis and blotting were done as described (26). DNA probes were radiolabeled with the Rediprime kit (Amersham), and hybridization to blots and detection of hybrids were done as described (26).

Blots were stripped before reprobing according to manufacturer's (ICN) recommendations.

The DNA fragment used as a *ctrA* probe was a *Hin*dIII–*Pvu*I fragment that contains from nucleotide 121 through the end of *ctrA* (≈600 bp) and 170 bp of *dnlJ*. The orfg2 probe was a *Eco*RI–*Hin*dIII fragment that contains ≈500 bp of orfg2.

Results

GTA Structural Genes. A *R. capsulatus* Y262::Tn5 library was screened for mutants that had lost the ability to produce GTA (see *Materials and Methods*). This procedure yielded four unique isolates that had lost the ability to produce detectable levels of GTA (GTA bioassays of mutant cultures resulted in no transductants whereas the parental strain, Y262, yielded 10^3–10^4 transductants). All four Tn5 insertions mapped to a 30-kb region of the chromosome. DNA sequence analysis of this area of the chromosome revealed ORFs in this region (Fig. 1), and database searches yielded homologs for most of these ORFs (Table 1). The DNA sequences used for the analysis were obtained from two strains. A 3,762-bp *Eco*RI fragment, which contains from 60 bp 5′ of orfg2 to 300 bp into orfg5, from strain B10 was sequenced (accession no. AF181080), as was a 796-bp fragment from strain B10 that contains the *ctrA* gene (accession no. AF181078). The remaining sequences used were of strain SB1003, obtained from http://rhodoL.uchicago.edu/capsulapedia/capsulapedia/capsulapedia.shtml. Comparison of the 4.5 kb of overlapping sequences from these two strains showed only one difference, located in orfg4: the presence of an extra "G" in the B10 sequence, which resulted in one continuous ORF homologous to a phage gene, whereas the SB1003 sequence encoded two smaller ORFs homologous to different parts of the same gene.

Three of the four initially characterized Tn5 insertions were located within a region of ≈15 kb that contains 19 ORFs with the same transcriptional polarity. Several ORFs within this cluster exhibit significant amino acid sequence similarity to known or putative phage and prophage proteins. The GTA orfg2 and orfg4 are most similar to head proteins of *E. coli* phages T4 and HK97, respectively; orfg3 and orfg5 are similar to head proteins from *Streptomyces spp*. phage φC31; orfg11 is similar in sequence to a gene from *Salmonella typhimurium* that is similar to a tail protein of the *E. coli* phage λ.

The N-terminal amino acid sequence of an ≈30-kDa protein from purified GTA particles was determined to be ALNSAV-AAEGGYLVDPQTSETIR. This matches amino acid residues 101–123 of the orfg5 sequence perfectly. On the basis of database search results, this ORF is predicted to be the major capsid protein. Thus, it appears that the full-length GTA capsid protein precursor is cleaved on the C-terminal side of a lysine residue to produce the mature capsid protein during maturation of the head structure, similar to phage HK97 (27).

Lang and Beatty

Table 1. Summary of database search results for predicted ORF products

ORF	Predicted size, amino acids	3' Distance to next ORF, bp*	Database match, accession no.	E value[†]
trmU	354	198	Probable tRNA methyltransferase, P73755	8×10^{-56}
II.1	92	97	ORF in flagellar gene cluster, AF072135	5×10^{-17}
ctrA	237	62	CtrA/response regulator, AF051939	3×10^{-87}
dnlJ	707	−4	DNA ligase, Z11910	1×10^{-166}
recG	680		RecG/recombination, X59550	1×10^{-123}
fabF	420	−4	KAS II/fatty acid synthesis, AJ235273	1×10^{-122}
chp	390	192	Conserved hypothetical protein, U32728	1×10^{-43}
orfg1	107	114	None	
orfg2	393	184	T4 DNA packaging protein, X52394	5×10^{-03}
orfg3	396	246	øC31 portal protein, AJ006589	2×10^{-15}
orfg4	184	31	HK97 prohead protease protein, U18319	3×10^{-08}
orfg5	398	171	øC31 capsid protein, AJ006589	2×10^{-12}
orfg6	179	48	None	
orfg7	112	−4	None	
orfg8	135	41	None	
orfg9	137	8	Antigen A, Y09161	2×10^{-03}
orfg10	108	211	None	
orfg11	219	11	λ Tail component homolog, AF007380	9×10^{-07}
orfg12	247	47	None	
orfg13	243	−4	None	
orfg14	150	1	Putative lipoprotein, D86610	8×10^{-05}
orfg15	346	82	None	
orfg16	100	103	None	
orfg17	113	87	None	
orfg18	651	93	Similar to rhamnosyl transferase, AL008967	4.5×10^{-02}
orfg19	69	60	None	
cysE	279		CysE/cysteine biosynthesis, X59594	4×10^{-69}

*Negative values indicate translational overlap.

[†]Number of equal scoring matches expected by random chance; results with a value $< 10^{-1}$ are included.

The database searches with orfg9, orfg14, and orfg18 produced low scoring matches of uncertain significance. Orfg9 is 20% identical over 131 aa to a secreted antigen from pathogenic strains of *Listeria monocytogenes*, but the function of this protein is unknown (28). The predicted product of orfg14 is 29% identical over 136 aa to a putative lipoprotein and therefore could interact with the inner and/or outer membranes of recipient cells. Orfg18 is 26% identical over a 204-aa stretch to the hypothetical protein Rv2739c from *Mycobacterium tuberculosis*, which in turn is similar in sequence to a rhamnosyl transferase. Because rhamnose is a component of the *R. capsulatus* capsule (29), we speculate that the orfg18 product may interact with the capsule of recipient cells.

Transcriptionally congruent insertions of the KIXX cartridge, which rarely produce polar mutations in *R. capsulatus* (30), were introduced into orfg2 and orfg4 (the putative terminase and protease genes, respectively) to confirm the specific requirement for these genes in GTA production. These insertions reduced GTA production to undetectable levels.
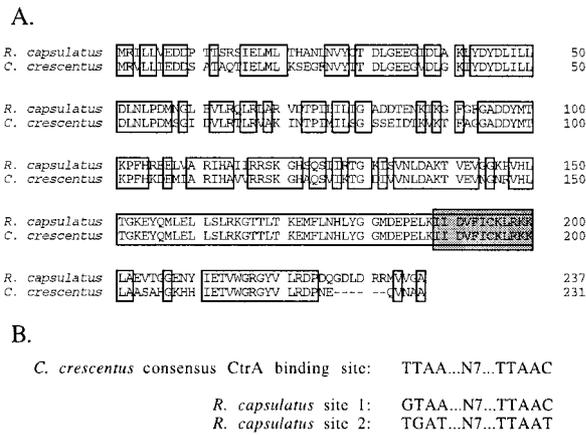
The database searches using orfg1–orfg19 indicate that GTA is a novel member of a family of double-stranded DNA phages (31). The order of phage-homologous GTA genes is the same as in phages from diverse genera of bacteria (31), and a high level of inter-phage amino acid sequence conservation exists in spite of a preferred high GC codon usage in GTA ORFs. The codon usage of most of the GTA ORFs matches highly expressed *R. capsulatus* genes well (data not shown), unlike previously described *R. capsulatus* prophages (32). We propose that the orfg1–orfg19 region is a GTA structural gene cluster flanked by cellular "housekeeping" genes. This cluster is flanked on the 3' side by a *cysE* (cysteine biosynthesis) homolog and on the 5' side by an ORF homologous to a widespread hypothetical bacterial

protein (*chp*) and a *fabF* (fatty acid biosynthesis) homolog. There are no other phage gene homologs within 90 kb of the GTA structural gene cluster.

GTA Regulatory Genes. The *R. capsulatus ctrA* gene is located 5' of *dnlJ* (DNA ligase) and *recG* (DNA recombination) homologs, and 3' of an ORF whose predicted product is similar to the II.1 protein from *C. crescentus*. A transcriptionally congruent insertion of the KIXX cartridge into *ctrA* (strain YCKF) reduced GTA production to an undetectable level, and so the *ctrA* gene seems to positively regulate GTA gene expression.

The *R. capsulatus* predicted CtrA amino acid sequence is 75% identical to the homolog from *B. abortus* and 71% identical to the *C. crescentus* CtrA protein (Fig. 2A). By alignment with the OmpR protein from *E. coli* (34) the putative DNA recognition helix of the *C. crescentus* CtrA protein was identified, and the *C. crescentus* and *R. capsulatus* sequences were found to be identical in this region (Fig. 2A). This result suggests that these two proteins recognize similar DNA sequences, and binding sites are known for the *C. crescentus* CtrA (35). There are two potential CtrA recognition sites 5' of *ctrA* in *R. capsulatus* (Fig. 2B) that could indicate auto-regulation of *ctrA* transcription if these are genuine CtrA-binding sites. No potential CtrA-binding sites were found 5' of the GTA gene cluster, which leads us to speculate that the *R. capsulatus* CtrA protein regulates the expression of another gene whose product, perhaps a σ factor, directly regulates GTA gene transcription.

The plasmid pSTU12 (orfg2'::'lacZ fusion) was conjugated into Y262::Tn5 library cells; the plasmid recipients were screened for colonies with weak β-galactosidase activity, and a fifth mutant that did not produce detectable levels of GTA was found. The Tn5 insertion in this mutant is located in a *R.*

A.

B.

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C. crescentus consensus CtrA binding site:    TTAA...N7...TTAAC

                        R. capsulatus site 1:    GTAA...N7...TTAAC
                        R. capsulatus site 2:    TGAT...N7...TTAAT
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Fig. 2. Comparisons of CtrA proteins and DNA binding sites. (*A*) Alignment of the CtrA amino acid sequences from *R. capsulatus* and *C. crescentus*. Identical amino acids (71%) are boxed, and the putative sequence specific DNA recognition domains of the proteins are shaded. (*B*) The consensus CtrA-binding site of *C. crescentus* compared with potential CtrA-binding sites located 5′ of the *R. capsulatus ctrA* gene. Sites 1 and 2 are ≈70 bp and 30 bp 5′ of the *ctrA* start codon, respectively.

capsulatus gene with a predicted amino acid sequence 44% identical to that of the CckA protein from *C. crescentus* over the 419 C-terminal codons of the *R. capsulatus* B10 gene that were sequenced (Fig. 3). The chromosomal location of this *R. capsulatus cckA* gene is not known, but it is not within 90 kb of the GTA gene cluster.

The CckA protein is required for the activation (by phosphorylation) of CtrA in *C. crescentus* (36), although it is unclear whether there is a direct interaction between these two proteins or if there are other proteins in a phosphorelay system (37). Thus it seems that the *R. capsulatus* CckA protein is involved in CtrA activation for GTA production.

Regulation of GTA Gene Expression. The strain Y262(pYP) (plasmid pYP contains an orfg2′::′*lacZ* fusion) exhibited growth-phase-dependent β-galactosidase activity, with a >4-fold increase in specific activity in the late stationary phase relative to
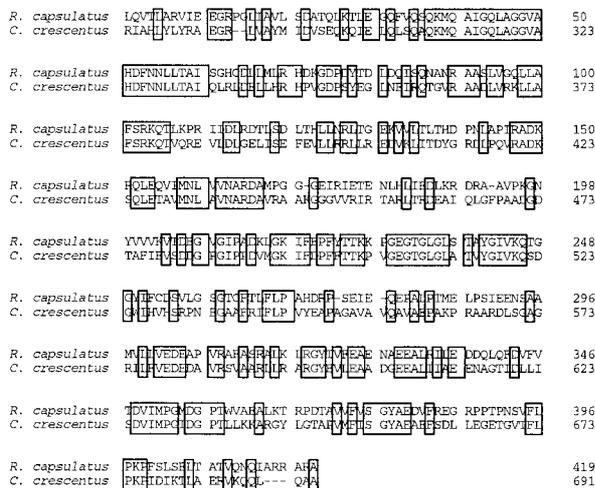
Fig. 3. Alignment of predicted CckA protein sequences of *R. capsulatus* and *C. crescentus*. The proteins are 44% identical over the sequence shown. Residues 274–691 of the *C. crescentus* protein (accession no. AF133718) are aligned with the available 419 aa of the *R. capsulatus* sequence (accession no. AF181079).

A.

B.
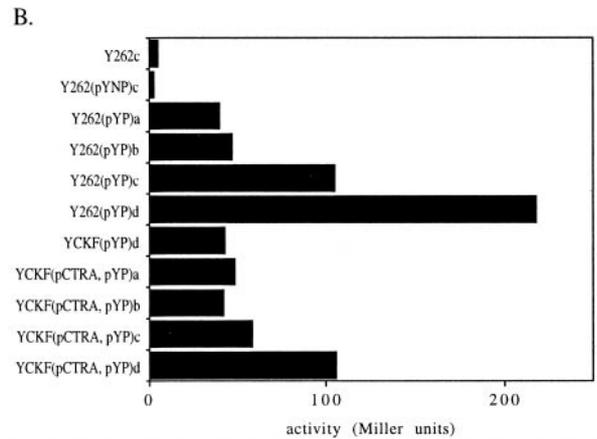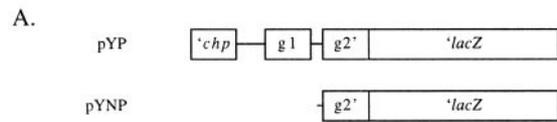
Fig. 4. Growth-phase and CtrA dependence of β-galactosidase activities of orfg2′::′*lacZ* plasmid-borne gene fusions. (*A*) Maps of the orfg2′::′*lacZ* fusions used to investigate GTA gene expression. (*B*) β-galactosidase specific activities. The cell samples indicated left of the graph designate: Y262, plasmid-free Y262; Y262(pYNP), Y262 containing plasmid pYNP; Y262(pYP), Y262 containing plasmid pYP; YCKF(pYP), YCKF containing plasmid pYP; and YCKF(pCTRA, pYP), YCKF containing plasmids pCTRA and pYP. Cells were harvested over the growth phase at a, mid-log phase; b, late-log phase; c, early stationary phase; and d, late stationary phase.

mid-log and late-log phases (Fig. 4). These data are consistent with the report that GTA production is maximal in the stationary phase (6). The *ctrA* mutant YCKF(pYP) produced a low level of β-galactosidase activity in the stationary phase, similar to mid-log and late-log phase Y262(pYP) cultures. Complementation of strain YCKF(pYP) with the *ctrA* gene on the compatible pCTRA plasmid partially restored the growth-phase-dependent β-galactosidase activity. These results confirm that CtrA is a positive regulator of GTA gene expression, perhaps at the level of transcription initiation. The activities obtained with the mid-log and late-log phase Y262(pYP) (*ctrA*⁺) and the stationary phase YCKF(pYP) (*ctrA*⁻) cultures indicate a basal level of CtrA-independent expression, whereas the increase in expression in the stationary phase was dependent on CtrA. It was surprising to find that YCKF(pYP) contained ≈20% of the β-galactosidase activity of that in Y262(pYP), as there was no detectable GTA production by strain YCKF. Although it might seem there could be an effect from the gene fusions being on a plasmid with a copy number (in *E. coli*) of 5–8 (38), such that the presence of several plasmid-borne binding sites for a GTA gene negative regulator increases the CtrA-independent expression level, GTA production was undetectable in strain YCKF when it contained plasmid pYP.

The differences in β-galactosidase activities obtained with the orfg2′::′*lacZ* fusions in Y262(pYP) and Y262(pYNP) show that a promoter required to transcribe orfg2, and presumably the other GTA structural genes, is located between the middle of *chp* and ≈50 bp 5′ of the start of orfg2 (a region of ≈900 bp), because Y262(pYNP) cells had the same low activity as plasmid-free cells (Fig. 4). Examination of the DNA sequence in the region delimited by pYP and pYNP revealed a sequence (5′-<u>AAAA-CCCCGG</u>CTTCGT<u>CCGGGGGTTTTTT</u>CTTTT-3′) that could encode a ρ-independent transcription terminator (39, 40) beginning 3 bp 3′ of the stop codon for *chp*, and 27 bp 3′ of this motif is a sequence (5′-TTGACT-N17-TAGAGAT-3′) that is

A. ctrA probe
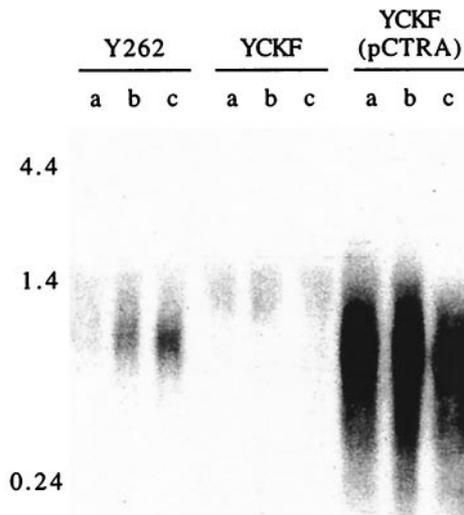
B. orfg2 probe
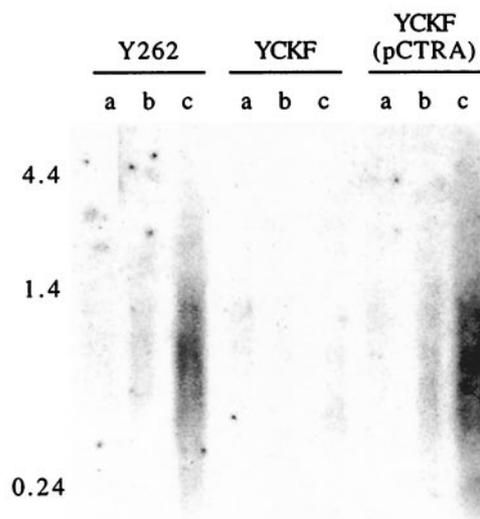
Fig. 5. RNA blot analysis of GTA gene transcripts. RNA was isolated from the strains indicated above the lines at three time points over the growth cycle: a, b, and c represent mid-log, late-log, and early stationary phases, respectively. Equal amounts (10 μg) of RNA were loaded in each lane. Approximate locations of molecular weight RNA standards are shown on the left (in kb). (*A*) Results when the blot was probed with a fragment of *ctrA*. (*B*) Results when the blot was probed with a fragment of orfg2.

similar to known σ factor-binding sites. The 5′-half of the latter sequence is similar to σ^{70} sites from *R. capsulatus* (41) and *E. coli* (42), and the 3′ portion is similar to the analogous region of a σ^{28} site from *Bacillus subtilis* (43). The most 3′ of these sequences is located ≈540 bp 5′ of the predicted start codon of orfg2.

RNA blot analysis directly confirmed that the levels of *ctrA* and orfg2 transcripts are growth-phase dependent (Fig. 5). The level of orfg2 messages was negligible except during the stationary phase, whereas *ctrA* RNA was weakly visible during the late-log phase and increased in amount in the stationary phase. There was a considerable size heterogeneity of RNA molecules complementary to the orfg2 probe but less heterogeneity of the

ctrA transcript. When this blot was stripped and reprobed with a segment of orfg4, the same growth-phase-dependent expression pattern as for orfg2 was seen (data not shown). The RNAs complementary to the orfg4 probe also showed size heterogeneity, but over a different size range than for orfg2, consistent with the idea that a primary transcript is rapidly degraded to yield a variety of RNA molecules encoding GTA proteins, analogous to the transcripts of phage ϕC31 (44, 45). When *ctrA* was disrupted (strain YCKF) there were no detectable orfg2 or orfg4 transcripts, which explains the absence of GTA production in *ctrA* mutants. Complementation of the *ctrA* mutant YCKF with pCTRA restored orfg2 and orfg4 transcripts and GTA production. Thus CtrA appears to be required for maximal transcription of GTA structural genes.

Despite constitutive *ctrA* transcription in the complemented $ctrA^-$ strain YCKF(pCTRA), GTA gene transcription was growth-phase dependent, as in strain Y262. This indicates that although CtrA is necessary, it is insufficient to promote optimal transcription of the GTA genes. Therefore, it seems that another cellular protein, perhaps CckA, activates CtrA to enhance GTA gene transcription in the stationary phase.

Discussion

We suggest that the orfg1–orfg19 region is an operon of GTA structural genes. This 15-kb GTA gene cluster is too small to encode a genuine phage, which by analogy with the phages HK97 (accession no. AF069529) and ϕC31 (46) that contain GTA gene homologs, would require a genome of ≈40 kb. There could be additional GTA structural genes in a separate locus that we did not find or, alternatively, the gene cluster shown in Fig. 1 is sufficient to encode GTA particles. Because GTA randomly packages 4.5-kb linear genomic DNA fragments (3–5), phage DNA-specific replication functions are not required to produce DNA destined for packaging. Similarly, phage DNA-specific integration and excision functions should not be required for gene transmission by GTA because GTA-dependent allelic replacement seems to be mediated by a cellular (*recA*-dependent) recombination system (47). There does not appear to be a GTA lysin/holin system, and GTA-induced cell lysis is undetectable (2). Our data show that control of GTA gene transcription is positively regulated by the cellular proteins CckA and CtrA. Therefore, the gene cluster shown in Fig. 1 could contain all the information needed for assembly of the GTA head and tail structures and DNA packaging. In the related phage ϕC31, transcription of the head and tail genes is driven by a single promoter (44, 45), and gene organization of the head–tail region is a highly conserved feature amongst double-stranded DNA phages (48). Sequence differences between the GTA genes and phage homologs evidently account for the differences in terms of quantity and quality of DNA that is packaged. However, the details of how 4.5-kb segments of genomic DNA are encapsidated by GTA and particles are released from cells are unclear.

Our discovery that disruption of the *R. capsulatus cckA* gene impaired GTA production, as did disruption of *ctrA*, strongly indicates that the proteins encoded by these genes are (part of) a sensor kinase/response regulator system that controls transcription of GTA genes in response to a stationary phase environmental signal. The nature of this signal and how this system came to regulate the expression of GTA genes begs for additional research. The *C. crescentus* CtrA and CckA proteins are required for viability and are involved in cell cycle transitions (49), flagellum development (35), DNA replication (35, 36, 50), DNA methylation (35), and cell division (33, 36). Our data show that neither CtrA nor CckA is required for viability of *R. capsulatus*, and although there might be an analogy between the *C. crescentus* cell cycle and the *R. capsulatus* growth phase, these two concepts are different.

The results presented in this paper indicate that there has been an evolutionarily long relationship between GTA and *R. capsulatus*. However, the question of whether GTA is a defective bacteriophage or a phage precursor remains. Because fewer assumptions would be required to posit a phage ancestor of GTA, as opposed to vice versa, the simplest interpretation is that GTA is a defective prophage. Regardless of its genesis, GTA is used by *R. capsulatus* as a genetic exchange vector, such that the production of GTA is controlled by a set of cellular proteins that activate the process of gene transfer in the stationary phase of growth of laboratory cultures. We suggest that, in nature, a response to growth-limiting environmental conditions causes a signal to be transduced through CckA, CtrA, and at least one additional protein to activate GTA gene expression. This results in the production of GTA particles that transduce genomic DNA fragments to other *R. capsulatus* cells, enhancing the genetic diversity of a population of cells confronted by a hostile environment.

1. Mazodier, P. & Davies, J. (1991) *Annu. Rev. Genet.* **25,** 147–171.
2. Marrs, B. L. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 971–973.
3. Solioz, M. & Marrs, B. (1977) *Arch. Biochem. Biophys.* **181,** 300–307.
4. Yen, H.-C., Hu, N. T. & Marrs, B. L. (1979) *J. Mol. Biol.* **131,** 157–168.
5. Scolnik, P. A. & Haselkorn, R. (1984) *Nature (London)* **307,** 289–292.
6. Solioz, M., Yen, H.-C. & Marrs, B. (1975) *J. Bacteriol.* **123,** 651–657.
7. Wall, J. D., Weaver, P. F. & Gest, H. (1974) *Arch. Microbiol.* **105,** 217–224.
8. Rapp, B. J. & Wall, J. D. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 9128–9130.
9. Humphrey, S. B., Stanton, T. B., Jensen, N. S. & Zuerner, R. L. (1997) *J. Bacteriol.* **179,** 323–329.
10. Bertani, G. (1999) *J. Bacteriol.* **181,** 2992–3002.
11. Brent, R. & Ptashne, M. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 1932–1936.
12. Simon, R., Priefer, U. & Pühler, A. (1983) *Bio/Technology* **1,** 37–45.
13. Taylor, D. P., Cohen, S. N., Clark, W. G. & Marrs, B. L. (1983) *J. Bacteriol.* **154,** 580–590.
14. Ditta, G., Schmidhauser, T., Yakobsen, E., Lu, P., Liang, X.-W., Finlay, D. R., Guiney, D. & Helinski, D. R. (1985) *Plasmid* **13,** 149–153.
15. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY).
16. Yen, H.-C. & Marrs, B. (1976) *J. Bacteriol.* **126,** 619–629.
17. Marrs, B. L. (1978) *Curr. Top. Bioenerget.* **8,** 261–294.
18. Marrs, B. (1981) *J. Bacteriol.* **146,** 1003–1012.
19. Wong, D. K.-H., Collins, W. J., Harmer, A., Lilburn, T. G. & Beatty, J. T. (1996) *J. Bacteriol.* **178,** 2334–2342.
20. Beatty, J. T. & Gest, H. (1981) *Arch. Microbiol.* **129,** 335–340.
21. Barany, F. (1985) *Gene* **37,** 111–123.
22. Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D. J. (1997) *Nucleic Acids Res.* **25,** 3389–3402.
23. Young, C. S., Reyes, R. C. & Beatty, J. T. (1998) *J. Bacteriol.* **180,** 1759–1765.
24. Adams, C. W., Forrest, M. E., Cohen, S. N. & Beatty, J. T. (1989) *J. Bacteriol.* **171,** 473–482.
25. Miller, J. H. (1992) *A Short Course in Bacterial Genetics: A Laboratory Manual and Handbook for Escherichia coli and Related Bacteria* (Cold Spring Harbor Lab. Press, Plainview, NY).
26. LeBlanc, H., Lang, A. S. & Beatty, J. T. (1999) *J. Bacteriol.* **181,** 4955–4960.
27. Duda, R. L., Martincic, K. & Hendrix, R. W. (1995) *J. Mol. Biol.* **247,** 636–647.
28. Schäferkordt, S. & Chakraborty, T. (1997) *J. Bacteriol.* **179,** 2707–2716.
29. Omar, A. S., Weekesser, J. & Mayer, H. (1983) *Arch. Microbiol.* **136,** 291–296.
30. Bollivar, D. W., Suzuki, J. Y., Beatty, J. T., Dobrowski, J. M. & Bauer, C. E. (1994) *J. Mol. Biol.* **237,** 622–640.
31. Hendrix, R. W., Smith, M. C. M., Burns, R. N., Ford, M. E. & Hatfull, G. F. (1999) *Proc. Natl. Acad. Sci. USA* **96,** 2192–2197.
32. Vlcek, C., Paces, V., Maltsev, N., Paces, J., Haselkorn, R. & Fonstein, M. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 9384–9388.
33. Kelly, A. J., Sackett, M. J., Din, N., Quardokus, E. & Brun, Y. V. (1998) *Genes Dev.* **12,** 880–893.
34. Martinez-Hackert, E. & Stock, A. M. (1997) *J. Mol. Biol.* **269,** 301–312.
35. Quon, K. C., Marczynski, G. T. & Shapiro, L. (1996) *Cell* **84,** 83–93.
36. Jacobs, C., Domian, I. J., Maddock, J. R. & Shapiro, L. (1999) *Cell* **97,** 111–120.
37. Wu, J., Ohta, N. & Newton, A. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 1443–1448.
38. Figurski, D. H., Meyer, R. J. & Helinski, D. R. (1979) *J. Mol. Biol.* **133,** 295–318.
39. Chen, C.-Y. A., Beatty, J. T., Cohen, S. N. & Belasco, J. G. (1988) *Cell* **52,** 609–619.
40. d'Auberton Carafa, Y., Brody, E. & Thermes, C. (1990) *J. Mol. Biol.* **216,** 835–858.
41. Nickens, D. G. & Bauer, C. E. (1998) *J. Bacteriol.* **180,** 4270–4277.
42. Hawley, D. K. & McClure, W. R. (1983) *Nucleic Acids Res.* **11,** 2237–2255.
43. Gilman, M. Z., Wiggs, J. L. & Chamberlin, M. J. (1981) *Nucleic Acids Res.* **9,** 5991–6000.
44. Howe, C. W. & Smith, M. C. (1996) *Microbiol.* **142,** 1357–1367.
45. Suarez, J. E., Clayton, T. M., Rodriguez, A., Bibb, M. J. & Chater, K. F. (1992) *J. Gen. Microbiol.* **138,** 2145–2157.
46. Smith, M. C. M., Burns, R. N., Wilson, S. E. & Gregory, M. A. (1999) *Nucleic Acids Res.* **27,** 2145–2155.
47. Genthner, F. J. & Wall, J. D. (1984) *J. Bacteriol.* **160,** 971–975.
48. Casjens, S., Hatfull, G. & Hendrix, R. (1992) *Semin. Virol.* **3,** 383–397.
49. Domian, I. J., Quon, K. C. & Shapiro, L. (1997) *Cell* **90,** 415–424.
50. Quon, K. C., Yang, B., Domian, I. J., Shapiro, L. & Marczynski, G. T. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 120–125.