JB
Journals.ASM.org

# Homologues of Genetic Transformation DNA Import Genes Are Required for *Rhodobacter capsulatus* Gene Transfer Agent Recipient Capability Regulated by the Response Regulator CtrA

**Cedric A. Brimacombe, Hao Ding, Jeanette A. Johnson, J. Thomas Beatty**

Department of Microbiology and Immunology, The University of British Columbia, Vancouver, British Columbia, Canada

**ABSTRACT**

**Gene transfer agents (GTAs) morphologically resemble small, double-stranded DNA (dsDNA) bacteriophages; however, their only known role is to package and transfer random pieces of the producing cell genome to recipient cells. The best understood GTA is that of *Rhodobacter capsulatus*, termed RcGTA. We discovered that homologues of three genes involved in natural transformation in other bacteria, *comEC*, *comF*, and *comM*, are essential for RcGTA-mediated gene acquisition. This paper gives genetic and biochemical evidence that RcGTA-borne DNA entry into cells requires the ComEC and ComF putative DNA transport proteins and genetic evidence that putative cytoplasmic ComM protein of unknown function is required for recipient capability. Furthermore, the master regulator of RcGTA production in <1% of a cell population, CtrA, which is also required for gene acquisition in recipient cells, is expressed in the vast majority of the population. Our results indicate that RcGTA-mediated gene transfer combines key aspects of two bacterial horizontal gene transfer mechanisms, where donor DNA is packaged in transducing phage-like particles and recipient cells take up DNA using natural transformation-related machinery. Both of these differentiated subsets of a culture population, donors and recipients, are dependent on the same response regulator, CtrA.**

**IMPORTANCE**

**Horizontal gene transfer (HGT) is a major driver of bacterial evolution and adaptation to environmental stresses. Traits such as antibiotic resistance or metabolic properties can be transferred between bacteria via HGT; thus, HGT can have a tremendous effect on the fitness of a bacterial population. The three classically described HGT mechanisms are conjugation, transformation, and phage-mediated transduction. More recently, the HGT factor GTA was described, where random pieces of producing cell genome are packaged into phage-like particles that deliver DNA to recipient cells. In this report, we show that transport of DNA borne by the *R. capsulatus* RcGTA into recipient cells requires key genes previously thought to be specific to natural transformation pathways. These findings indicate that RcGTA combines central aspects of phage-mediated transduction and natural transformation in an efficient, regulated mode of HGT.**

The first evidence of prokaryotic genetic exchange was transformation, a term coined in 1928 by Griffith (1). Subsequently, conjugation was observed in 1946 (2), followed by transduction, which was discovered in 1952 (3). More recently, another prokaryotic mode of horizontal gene transfer, dependent on an extracellular particle called a gene transfer agent (GTA), was described (4), and GTAs subsequently have been discovered in diverse prokaryotes (5).

GTAs varying in morphology have been reported, although most resemble small, tailed double-stranded DNA (dsDNA) bacteriophages. The general criteria that define a GTA are (i) the DNA packaged within the head is insufficient to carry the GTA structural genes; (ii) all GTA particles package only random parts of the producing cell's genome (as opposed to packaging of host cell genomic DNA in generalized transducing phages, which is very infrequent); and (iii) production is controlled by cellular regulatory systems (5–7). As a consequence, the frequency of cellular gene transduction by GTAs is much greater than that by generalized transducing phages.

The best-understood GTA, found in the alphaproteobacterium *Rhodobacter capsulatus*, is called RcGTA. RcGTA particles morphologically resemble a small, siphoviridae-like bacteriophage (8), and RcGTA structural gene organization and sequence composition are similar to those of genuine bacteriophages (5). However, nucleic acid analyses revealed that essentially random ~4-kb linear, dsDNA fragments of the producing cell genome with 3′ overhangs are packaged within particles (7, 8), so genetic markers are readily transferred from donor to recipient cells (5, 6). The RcGTA primary structural gene cluster consists of an ~15-kb region of the chromosome, and additional essential factors, including a holin and endolysin, are encoded in distant genome regions (5, 7, 9). Production of RcGTA requires the CtrA response regulator, and transcription is modulated by the GtaI/R quorum-sensing system (10–12). The ability of cells to receive an RcGTA-

**Received** 29 April 2015 **Accepted** 27 May 2015

**Accepted manuscript posted online** 1 June 2015

**Citation** Brimacombe CA, Ding H, Johnson JA, Beatty JT. 2015. Homologues of genetic transformation DNA import genes are required for *Rhodobacter capsulatus* gene transfer agent recipient capability regulated by the response regulator CtrA. J Bacteriol 197:2653–2663. doi:10.1128/JB.00332-15.

**Editor:** P. de Boer

Address correspondence to J. Thomas Beatty, j.beatty@ubc.ca.

Supplemental material for this article may be found at http://dx.doi.org/10.1128/JB.00332-15.



doi:10.1128/JB.00332-15

**TABLE 1** Plasmids and strains used and/or generated in this study

| Plasmid or strain | Feature(s)/inserted sequence[a] or description | Reference or source |
|---|---|---|
| Plasmids | | |
| pUC19Fup | *comF* upstream flanking region ligated into pUC19 | This study |
| pUC19Fdown | *comF* downstream flanking region ligated into pUC19 | This study |
| pUC19Mup | *comM* upstream flanking region ligated into pUC19 | This study |
| pUC19Mdown | *comM* downstream flanking region ligated into pUC19 | This study |
| pUC19EC | *comEC* gene and flanking regions ligated into pUC19 | This study |
| pComF | ~500 bp 5′ of *comF* gene, the *comF* gene, and ~200 bp downstream ligated into pCM62 | This study |
| pComM | ~500 bp 5′ of *comM* gene, the *comM* gene, and ~200 bp downstream ligated into pCM62 | This study |
| pComEC | ~500 bp 5′ of *comEC* gene, the *comEC* gene, and ~200 bp downstream ligated into pCM62 | This study |
| pZDJΔ*comF* | Upstream and downstream flanks of *comF* fused together in pZDJ | This study |
| pZDJΔ*comM* | Upstream and downstream flanks of *comM* fused together in pZDJ | This study |
| pZDJ | $Gm^r$; Gram-negative suicide vector; contains *sacB* gene and *tetR* promoter | 27 |
| pUC19 | $Amp^r$ LacZα; gene cloning vector | Invitrogen |
| pCM62 | $Tc^r$ LacZα; broad-host-range vector | 29 |
| pCMmCherry | mCherry gene ligated into pCM62 | 13 |
| pZDJΔ1081 | *rcc01081* knockout construct; used in HR expt | 27 |
| pCtrAmCherry | *ctrA* translationally fused to mCherry in pCMmCherry | This study |
| *R. capsulatus* strains | | |
| B10 | Wild-type isolate | 4 |
| DE442 | $Rif^r$; RcGTA overproducer; believed to originate from Y262 | 8 |
| Δ*comF* mutant | Markerless deletion of *comF* gene; B10 background | This study |
| Δ*comEC* mutant | $Kan^r$; KIXX cassette inserted 1,012 bp into the *comEC* gene in forward direction; B10 background | This study |
| Δ*comM* | Markerless deletion of *comM* gene; B10 background | This study |
| DE442(pd1080Gm) | $Rif^r$ $Gm^r$; RcGTA overproducer; harbors plasmid pd1080Gm | Westbye, unpublished |

[a] Where applicable, the inserted sequence is described briefly.

carried genetic marker also is regulated by CtrA and quorum sensing, and in recipient cells DNA recombination occurs via a RecA/DprA recombination mechanism as in natural transformation systems (13).

The phrase "natural genetic competence" is defined as a physiological state in which bacteria actively take up exogenous DNA that may be recombined into the cell genome (14). The term "transformation" refers to the successful acquisition of a new genetic trait by recombination into the genome (14). It is now clear that natural competence systems are widespread in both Gram-positive and Gram-negative bacteria, and new functional systems are continually being discovered (14). Although some details differ, the general mechanisms of natural competence systems are the following. Exogenous DNA is bound by a pilus structure that brings DNA into proximity of the cytoplasmic membrane (the inner membrane in Gram-negative bacteria). DNA is thought to be transported through the membrane by the ComEC DNA transporter in a process also involving ComF, resulting in a single-stranded DNA (ssDNA) molecule entering the cytoplasm (14–16). The ssDNA is bound by the DprA protein, which facilitates the formation of RecA filaments on the ssDNA, and recombination with the recipient cell genome if sequence similarity exists (17, 18). In addition, the *comM* gene is required for maximal *Haemophilus influenzae* transformation, although the function is unknown (19).

In a previous report, it was shown that the RcGTA recipient capability (the capability of an *R. capsulatus* cell to receive an RcGTA-borne genetic marker) requires the CtrA response regulator, and that expression of homologues of natural competence genes *dprA*, *comEC*, *comF*, and *comM* is modulated by CtrA. Genetic analysis showed that one of these genes, *dprA*, is essential for RcGTA recipient capability, as is the RecA homologue (13). These findings indicated that RcGTA-carried DNA recombines into the chromosome of recipient cells via a natural transformation-like mechanism rather than one resembling temperate phages (20, 21). However, it was unknown whether three other homologues of natural competence genes downregulated in a *ctrA* mutant, namely, *comEC*, *comF*, and *comM*, were involved in RcGTA recipient capability (13).

Here, we provide genetic and biochemical evidence that *comEC* and *comF* facilitate uptake of RcGTA-delivered DNA and are required for recipient capability. Additionally, it was found that *comM* is required for recipient capability. We present a model in which RcGTA releases DNA into the periplasm of the recipient cell, which then is transported into the cytoplasm by the ComEC/ComF machinery in the same fashion as that in natural competence pathways.

## MATERIALS AND METHODS

**Bacterial strains and growth conditions.** The *Escherichia coli* strains DH5α λ*pir*, S17-1 λ*pir* (22), and TEC5 (23) were used for gene cloning and conjugation of plasmids into *R. capsulatus*. *E. coli* strains were grown at 37°C in LB medium (24) supplemented with the appropriate antibiotics at the following concentrations (in micrograms per milliliter): ampicillin, 150; gentamicin sulfate, 10; kanamycin sulfate, 50; tetracycline hydrochloride, 10.

*R. capsulatus* strains, listed in Table 1, were grown at 30°C in RCV defined medium (25) aerobically with shaking at 200 rpm for recipient capability, conjugation, and growth rate assays or were sealed in test tubes phototrophically for UV sensitivity and RcGTA tracking assays. For production of gentamicin-resistant ($Gm^r$) RcGTA in the tracking assay, cultures of the RcGTA overproducer strain DE442(pd1080::Gm) were grown phototrophically in YPS complex medium (26). Cells were harvested in

**TABLE 2** Primers used in this study

| Primer | 5′–3′ sequence[a] | Product | Restriction cut site |
|---|---|---|---|
| comF_up_for | GCTAGAGCTCGAACAATATCCTTGC | pUC19Fup | SacI |
| comF_up_rev | GCTAGAATTCCACCCGCAAGGCCGC | pUC19Fup | EcoRI |
| comF_down_for | GCATGAATTCTCGGTCGCGGTTCTG | pUC19Fdown | EcoRI |
| comF_down_rev | CTTATCTAGACGGTCCGAGGACAGA | pUC19Fdown | XbaI |
| comM_up_for | GTACGAGCTCAACCATTGCGAAACG | pUC19Mup | SacI |
| comM_up_rev | TACAGGATCCGAAAGCCACCGTATA | pUC19Mup | BamHI |
| comM_down_for | GCACGGATCCTCGCCGATCTGGACG | pUC19Mdown | BamHI |
| comM_down_rev | GACATCTAGACGGCACCAATGTCGC | pUC19Mdown | XbaI |
| JAB-rec-2-fwd | GTGATCCGGCTCACTCTAGATCAGGTGCG | pUC19EC | XbaI |
| JAB-rec-2-rev | GGCAGGCGTTTTCAAGCTTGTCCAGGG | pUC19EC | HindIII |
| comF_screen_for | GCGGCGTCGTCGTGC | NA[b] | NA |
| comF_screen_rev | TGCGGGTGGTGTAGATT | NA | NA |
| comM_screen_for | GGATCTAGGCTACTG | NA | NA |
| comM_screen_rev | TCCTGAGCGATGATGC | NA | NA |
| comF_comp_for | TACAGAGCTCCGCCATAGCTGACCC | pComF | SacI |
| comF_comp_rev | CAGTAAGCTTGGTGTAGATTTCGAC | pComF | HindIII |
| comM_comp_for | CGTAGAGCTCCGCTGGCTCGCTTCGG | pComM | SacI |
| comM_comp_rev | CGTAGGATCCCCGAGGTGCTGCTGC | pComM | BamHI |
| comEC_comp_for | GTACGAGCTCCTCCTGGGTGGCGA | pComEC | SacI |
| comEC_comp_rev | TAGTAAGCTTCTCGTCGAGGATCTG | pComEC | HindIII |
| gm_for | GTTATGGAGCAGCAACGA | NA | NA |
| gm_rev | GGAGTAGGTGGCTAC | NA | NA |
| pctrA_F | AGAGAAGCTTCTCTGGTCGCGCATCGG | pCtrAmCherry | HindIII |
| pctrA_R | AGAGGGATCCATCCTGGGTTCTCCGCA | pCtrAmCherry | BamHI |
| Gm_qPCR_for | GGTGGCTCAAGTATGGGCAT | NA | NA |
| Gm_qPCR_rev | CGAAAAGATCAAGAGCAGCCC | NA | NA |
| puhA_qPCR_for | AACGACGACGGCAAGCTCT | NA | NA |
| puhA_qPCR_rev | GCTTCTTCGTTCTCGACCGA | NA | NA |

[a] Restriction cut sites (if applicable) are underlined.
[b] NA, not applicable.

the stationary phase of growth. When appropriate, media were supplemented with the following (in micrograms per milliliter): gentamicin sulfate, 3; kanamycin sulfate, 10; rifampin, 80; and tetracycline hydrochloride, 1.

The optical density at 650 nm ($OD_{650}$) was used as a measure of the number of *R. capsulatus* colony-forming units per milliliter; an $OD_{650}$ of 1 is ~$4.5 \times 10^8$ CFU $ml^{-1}$.

**Recombinant DNA techniques, plasmids, and PCR primers.** Standard methods of DNA purification, restriction enzyme digestion, and other modification techniques were used (24). All plasmids and primer sequences used in this study are listed in Tables 1 and 2, respectively. The plasmid pUC19 was used for subcloning and pCM62 as a complementation plasmid.

**Creation of the Δ*comF* (*rcc197*) and Δ*comM* (*rcc460*) mutant strains.** The Δ*comF* mutant we generated is a markerless in-frame deletion of ~80% of the gene and was constructed from the wild-type (WT) strain B10. Approximately 900-bp (upstream) and 450-bp (downstream) flanking regions of the *comF* gene were PCR amplified as SacI-EcoRI (upstream) and EcoRI-XbaI (downstream) fragments and inserted into pUC19, generating the pUC19Fup and pUC19Fdown plasmids. The resultant plasmids were digested with the appropriate enzymes and SacI-EcoRI and EcoRI-XbaI fragments were inserted into the suicide plasmid pZDJ, generating pZDJΔ*comF*. This plasmid was conjugated into the WT strain B10 and allowed to recombine into the chromosome, selected for by acquisition of gentamicin resistance. Cells that underwent a second recombination event, resulting in the loss of the plasmid, were selected by aerobic growth on RCV agar medium containing 7% sucrose and screened for the loss of $Gm^r$. Replacement of the WT *comF* with the Δ*comF* allele was confirmed by PCR amplicon size and DNA sequencing using the primers comF_seq_for and comF_seq_rev. The Δ*comM* mutant was constructed in the same fashion, with approximately 1,000-bp flanking regions (for both upstream and downstream) of the *comM* gene amplified and ultimately subcloned as SacI-BamHI (upstream) and BamHI-XbaI (downstream) fragments into the suicide plasmid pZDJ, generating pZDJΔ*comM*. The steps thereafter were identical to those described for the construction of the Δ*comF* mutant, except the primers comM_seq_for and comM_seq_rev were used to evaluate *comM* allele size and for DNA sequencing. For a description of the pZDJ suicide plasmid, see reference 27.

**Creation of the Δ*comEC* (*rcc02368*) mutant strain.** The open reading frame (ORF) encoding ComEC (*rcc02362*) was amplified by PCR from the genome of WT strain B10 as an XbaI-to-HindIII fragment, using the primers JAB-rec-2-Fwd and JAB-rec-2-Rev. The amplified product was cloned into plasmid pUC19, and the gene was disrupted by insertion of a kanamycin resistance-encoding ~1.4-kb SmaI-KIXX cartridge (28) into the unique NruI restriction site within the *comEC* coding region (1,012 bp 3′ of the start codon). Both orientations of the SmaI-KIXX cartridge were obtained and confirmed by DNA sequencing. Both constructs were transformed into *E. coli* TEC5 and conjugated into the RcGTA overproducer strain DE442. Mutant strains were generated by RcGTA-mediated transfer of the disrupted versions of the genes into the chromosome of WT strain B10 (4). PCR using the original amplification primers and restriction endonuclease analysis were used to confirm the resultant kanamycin-resistant strains. Strains containing the KIXX cartridge in both orientations had an identical phenotype (data not shown), and results from the mutant strain with KIXX inserted in the forward direction (the direction of transcription of the KIXX *neo* gene is the same as that of the disrupted *comEC* gene) are used in this report.

**Complementation of *comEC*, *comF*, and *comM* mutants.** The complementation plasmids pComEC, pComF, and pComM were generated by amplifying the *R. capsulatus comEC*, *comF*, and *comM* genes and ~450 bp 5′ of the start codons and ~100 bp 3′ of the stop codons as SacI-

HindIII, SacI-HindIII, and SacI-BamHI fragments, respectively. The resultant amplicons were ligated into the vector pCM62 (29) and verified by DNA sequencing. The resultant plasmids, pComEC, pComF, and pComM, were conjugated into the Δ*comEC*, Δ*comF*, and Δ*comM* strains, respectively, to generate the native promoter-driven *trans*-complemented strains.

**Fluorescence microscopy.** Approximately ~1 kb of DNA sequence 5′ of the *ctrA* start codon was PCR amplified using primers pctrAF and pctrAR and cloned as a HindIII-BamHI fragment into pCM62::mCherry (13), resulting in an in-frame fusion of the *ctrA* start codon to the mCherry coding region. The resultant construct (pCtrAmCherry) was conjugated into *R. capsulatus* B10 WT cells and evaluated for fluorescence. Images of cells grown in RCV medium to the stationary phase at 30°C were taken using a Sony DSC-S75 digital camera mounted on a Zeiss Axioskop fluorescence microscope equipped with a ×1,000 magnification objective. A mercury lamp (HBO 50X) and Zeiss filter set 15 (excitation, 546 nm and 12 nm; emission, 590 nm) were used to observe mCherry fluorescence.

**RcGTA-borne DNA tracking assay.** An RcGTA stock was prepared from a DE442 overproducer mutant harboring a plasmid containing the *rcc01080* gene (pd1080Gm) interrupted by a Gm$^r$ cassette (A. Westbye, unpublished data). This RcGTA stock was used because the Gm$^r$ gene is not present in the WT and mutant *R. capsulatus* strains studied in our work; thus, it can be tracked by PCR analysis from an extracellular location through periplasmic delivery to entry into the cytoplasm. For the tracking assay, WT, Δ*comEC*, and Δ*comF* strains were grown phototrophically in RCV medium to the stationary phase at 30°C. All harvesting and wash steps were done at room temperature (~22°C). Cells were harvested by centrifugation and suspended at an $OD_{650}$ of 3.0 in G-buffer (10 mM Tris-HCl [pH 7.8], 1 mM $MgCl_2$, 1 mM $CaCl_2$, 1 mM NaCl, 500 μg/ml bovine serum albumin [BSA]). A 500-μl sample of RcGTA stock and 500 μl of cells were mixed together and incubated at 30°C with gentle shaking for 60 min (for the no-GTA control, 500 μl of G-buffer and 500 μl of WT cells were mixed together). After the 60-min incubation, cells were harvested by centrifugation and washed three times in 1 ml of RCV medium. Cells then were suspended in 1 ml of RCV and incubated at 30°C with gentle shaking for an additional 120 min (for the 3-h time point and quantitative PCR [qPCR] measurements) or 23 h. At this point, 50 μl of each culture was plated onto RCV plus Gm to determine the Gm$^r$ colony-forming units per milliliter of each reaction, and the remaining cells were collected by centrifugation, washed 5 times with 1 ml of RCV medium, suspended in 400 μl of 50 mM EDTA, and incubated at 37°C for 60 min to disrupt the outer membrane (OM) and release periplasmic contents (30). Cells were pelleted by centrifugation to yield a cellular fraction, and the supernatant liquid (containing OM and periplasmic contents) was transferred to a fresh tube. DNA was extracted from the OM/periplasmic and cellular fractions by phenol-chloroform extraction, followed by isopropanol precipitation, and OM/periplasmic DNA was dissolved in 30 μl of distilled water ($dH_2O$), whereas cellular (chromosomal) DNA preparations were dissolved in 500 μl of $dH_2O$. Wash efficacy was evaluated by taking samples after each wash step and determining the presence of RcGTA-borne DNA by PCR using the primers Gm_qPCR_for and Gm_qPCR_rev. RcGTA-borne PCR amplification was undetectable after ~5 washes (see Fig. S3 in the supplemental material).

To quantify the relative amount of RcGTA-borne DNA in the OM/periplasmic fraction, qPCR was used with primers specific for a donor allele, with cells taken after 3 h of incubation with RcGTA. The primers GmqPCR_for and GmqPCR_rev were used to detect the relative amounts of the Gm$^r$ gene in each periplasmic/OM fraction; these primers amplify an 85-bp region within the Gm$^r$ cartridge. Residual periplasm/OM DNA levels were normalized to the level of the chromosomal *puhA* gene (*rcc00659*) in the chromosomal DNA fraction of each sample. The *puhA* gene is an endogenous photosynthesis gene present in *R. capsulatus*; the primers puhA_qPCR_for and puhA_qPCR_rev were used for quantification. The SYBR select master mix (Applied Biosystems) was used per the manufacturer's instructions. Reaction mixtures of 20 μl containing SYBR select master mix, 400 nM specific primers, and the target template were used to quantify target DNA in an Applied Biosystems StepOne plus real-time PCR system, using the following program: 50°C for 2 min, followed by 40 cycles of 95°C for 15 s, 60°C for 15 s, and 72°C for 60 s (amplification), and then 95°C for 15 s, 60°C for 15 s, and 95°C for 15 s (melting curve). Amplicons were clearly distinguishable from primer dimers based on melting curve analysis. Amplification of targets occurred from 15 to 25 cycles. As negative controls, reaction mixtures containing no DNA template were always included. As positive controls, standard concentrations of DNA containing either the Gm$^r$ cartridge or *puhA* were included in all qPCR runs and were used to quantify the amount of DNA present in each test sample. One microliter of each DNA sample was used as the template in all qPCRs, and values are presented as the ratio of residual periplasmic/OM Gm$^r$ cartridge-containing DNA to the level of *puhA* DNA in the chromosomal fraction of cells. Additional control experiments, such as evaluation of wash stringency, are presented in Fig. S3 in the supplemental material. Standard curves for both the Gm cassette and *puhA* primer sets are given in Fig. S4 in the supplemental material, and the raw qPCR data are given in Table S6 in the supplemental material.

**RcGTA recipient capability assay.** The rifampin-resistant (Rif$^r$) RcGTA overproducer strain DE442 was the source of RcGTA for recipient capability and adsorption experiments. The titer of a sample of stationary-phase culture grown phototrophically in YPS complex medium, passed through a 0.2-μm-pore-diameter filter, was determined for RcGTA activity, and a diluted stock solution that produced ~800 Rif$^r$ transductants per 100 μl of donor filtrate was used. All strains used as RcGTA recipients were derived from the WT strain B10. Recipient cultures were grown aerobically in RCV defined medium with shaking at 200 rpm to the stationary phase, harvested, and resuspended in an equal volume of G-buffer. A transduction assay then was performed in which 100 μl of RcGTA stock, 100 μl of recipient cells, and 400 μl of G-buffer were mixed together. This mixture was incubated for 90 min at 30°C with gentle agitation, after which 900 μl of RCV medium was added, followed by incubation under the same conditions for 3 h. Cells were spread on RCV plates containing rifampin and incubated aerobically for 3 days. The number of Rif$^r$ colonies was counted, and the average was corrected by subtracting the number of spontaneous Rif$^r$ colonies (no addition of RcGTA; usually less than 3%). Because of variability in total numbers of transductants between individual experiments, RcGTA recipient capability efficiencies are normalized to the WT control in each experiment.

**RcGTA adsorption assay.** To quantitatively measure the ability of different strains to bind RcGTA, cultures were grown aerobically in RCV defined medium with shaking at 200 rpm to the stationary phase, and cells were harvested and resuspended at the same concentration in G-buffer. One hundred microliters of RcGTA solution (the same as the amount used in the RcGTA recipient capability assay), 100 μl of recipient cells, and 400 μl of G-buffer were mixed and incubated for 90 min at 30°C with gentle agitation, after which the mixture was passed through a 0.2-μm filter. The number of RcGTA particles in the filtrate (RcGTA that did not adsorb to cells) was quantified by using 100 μl of this filtrate as the RcGTA donor and WT strain B10 cells as the recipient. As controls, an assay that included no recipient cells for adsorption (no cells) and an assay that contained no RcGTA also were performed.

**Plasmid conjugation and HR frequency.** Stationary-phase *R. capsulatus* cells were harvested by centrifugation and suspended at an $OD_{650}$ of 1.0 in fresh RCV medium. *E. coli* strain S17-1 λ*pir*, containing the suicide plasmid pZDJΔ1081, was grown overnight (to the stationary phase) and harvested by centrifugation. Cells were washed three times in RCV medium and suspended in RCV medium at an $OD_{600}$ of 1.0. A 2:1 mixture of *R. capsulatus*-to-*E. coli* cells was spotted onto RCV agar plates and incubated at 30°C overnight. Mating spots then were suspended in 500 μl of RCV medium, and dilutions plated on RCV agar with or without gentamicin were incubated at 30°C for 3 days. The number of colonies was counted, and the homologous recombination (HR) frequency was calcu-

**TABLE 3** Predicted and verified RcGTA recipient capability genes[e]

| *R. capsulatus* gene[e] | Annotation | Predicted/verified function(s) and/or characteristic(s) of gene product | WT/Δ*ctrA* ratio | | Predicted TMS (*n*) | Phenotype of mutant(s) | |
|---|---|---|---|---|---|---|---|
| | | | Stationary[a] | Log[b] | | Transformation[c] | RcGTA recipient capability[d] |
| *rcc00197* | *comF* | Unknown; required for DNA transport from periplasm to cytoplasm | 3.23 | 5.35 | 1 | Not detectable | Unknown |
| *rcc00460* | *comM* | Unknown; putative ATPase and helicase-like domains | 2.91 | 1.89 | 0 | Variable; 0– to 300-fold reduction | Unknown |
| *rcc02362* | *comEC-rec2* | Integral membrane protein; transports DNA from periplasm to cytoplasm | 4.88 | 2.67 | 6 | Not detectable | Unknown |
| *rcc03098* | *dprA* | Recombination mediator; loads RecA into ssDNA | 9.38 | 3.75 | 0 | Not detectable | Not detectable |
| *rcc01751* | *recA* | Recombination protein A; RecA; DNA repair and homologous recombination | 1.47 | 1.22 | 0 | Not detectable | Not detectable |
| *rcc01663* | *ctrA* | Cell cycle transcriptional regulator CtrA; regulates transcription of RcGTA and *com* genes | 0.94 | 1.04 | 0 | Unknown | Not detectable |

[a] Ratio of WT to Δ*ctrA* microarray expression values for cells grown to the exponential phase of growth.
[b] Ratio of WT to Δ*ctrA* microarray gene expression values for cells grown to the stationary phase of growth.
[c] A $10^7$-fold reduction in transformation was used as the limit of detection in other species.
[d] A $10^6$-fold reduction in recipient capability was used as the limit of detection in *R. capsulatus*.
[e] Shown are predicted and verified RcGTA recipient capability genes, their annotations, predicted or verified functions, expression levels in the Δ*ctrA* mutant versus the wild type in both stationary and log phases, the numbers of predicted transmembrane segments (TMS), the phenotypes in natural transformation systems, and the phenotypes resulting from mutations in these genes in natural transformation systems and in RcGTA recipient capability. Microarray expression values are derived from data reported in reference 61, and Δ*dprA*, Δ*recA*, and Δ*ctrA* mutant phenotypes were reported previously by Brimacombe et al. (13).

lated by dividing the number of antibiotic-resistant colonies by the total number of colony-forming units.

**UV sensitivity assays.** *R. capsulatus* cultures were grown phototrophically in RCV defined medium at 30°C to late log phase and diluted in 10-ml portions of fresh RCV to ~$10^4$ CFU $ml^{-1}$. Diluted cultures were exposed to a 15-W germicidal lamp at a distance of 50 cm for 0, 5, 15, or 30 s as indicated, with gentle mixing. At each time point, 100-μl samples were taken, plated onto RCV agar in serial dilutions, and grown at 30°C for 4 days. The percent survival was calculated as the number of CFU arising from UV-treated samples divided by the number of CFU from the non-exposed cells.

**Bioinformatic analyses.** The predicted amino acid sequence of *R. capsulatus* ComEC (encoded by *rcc02362*), ComF (encoded by *rcc00197*), ComM (encoded by *rcc00460*), and DprA (encoded by *rcc03098*) were used to probe bacterial genomes for the presence of homologues using BLASTP (31). Hits in other organisms then were verified to be reciprocal best hits to the *R. capsulatus* homologues, and full-length alignments of *R. capsulatus* proteins and homologues using EMBOSS Needle pairwise sequence alignment software (32) were done to verify that proteins are genuine homologues. Phylogenetic trees of ComM and DprA homologues were generated using MUSCLE MSA alignments and a neighbor-joining algorithm (33, 34). Domain analysis of proteins was performed using Pfam (35).

## RESULTS

**Bioinformatic analysis of *comEC*, *comF* and *comM*.** We began our study by analyzing the amino acid sequence of ComEC, ComF, and ComM predicted proteins. ComEC is essential for DNA transport through the cytoplasmic membrane (CM) in all natural transformation systems studied to date, including the well-studied Gram-positive *Streptococcus pneumoniae* and *Bacillus subtilis* and Gram-negative *Haemophilus influenzae* and *Vibrio cholerae* (14, 16). The ComEC protein family members are predicted to contain six transmembrane segments (TMS), which is supported by *in vitro* studies of the *B. subtilis* protein (16). Hydropathy analysis of *R. capsulatus* ComEC (*rcc02362*) predicts six TMS, that the protein is located in the CM, and that it contains the two conserved domains of ComEC proteins, pfam03772 and DUF4131 (Table 3 and Fig. 1A). Because these features are present in genuine ComEC proteins, we hypothesized that *R. capsulatus rcc02362* encodes a ComEC.

Although the ComF mechanism is unknown, it appears to be involved in DNA transport through the CM, because *comF* null mutants have the same phenotype as *comEC* null mutants (19, 36). Hydropathy analysis showed that the *R. capsulatus* ComF homologue has one putative TMS near the C terminus, with the bulk of the protein being cytoplasmic (Table 3). Of two putative cytoplasmic domains of ComF, one is homologous to a phosphoribosyltransferase domain often involved in nucleotide salvage pathways (37), and the other is homologous to a double zinc ribbon domain often involved in DNA binding (38) (Fig. 1B). Therefore, it is possible that ComF is involved in a DNA processing step associated with DNA import into the cell via ComEC (19, 36).

A *comM* gene has been studied in *H. influenzae*; its expression is induced during competence, and it is required for maximal transformation efficiency (19, 39). The function of ComM proteins is not known; however, our bioinformatics analysis yielded some clues. The *R. capsulatus* ComM is predicted to be cytoplasmic (Table 3), and several regions of the protein share homology with an AAA+ ATPase (subunit I of $Mg^{2+}$ chelatase) (40). ComM also contains domains with weak similarity to RuvB, a Holliday junction DNA helicase (41), and an MCM2/3/5 (Fig. 1C), which are helicases in the initiation of DNA replication in archaea and eukaryotes (42). We also observed that ComM homologues are not ubiquitous in the genomes of naturally transformable bacteria (see Fig. S1 in the supplemental material). Instead, ComM homologues are detectable only in genomes in which the corresponding

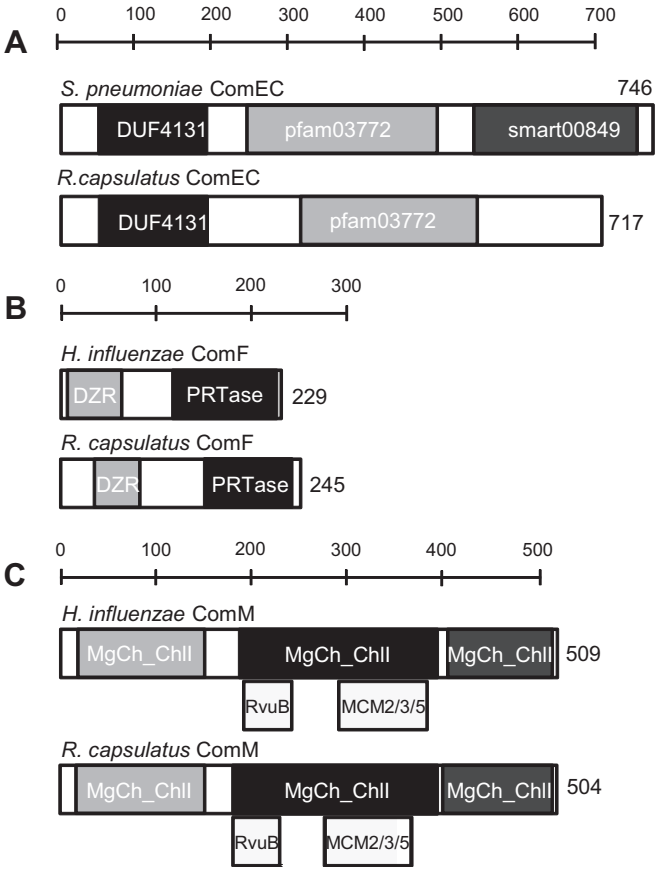


**FIG 1** Summary of bioinformatic analyses of *R. capsulatus* ComEC, ComF, and ComM proteins. (A) Comparison of *S. pneumoniae* ComEC to *R. capsulatus* ComEC. (B) Comparison of *H. influenzae* ComF to *R. capsulatus* ComF. (C) Comparison of *R. capsulatus* ComM to *H. influenzae* ComM. Amino acid numbers and domains are indicated. DUF4131 is a functionally uncharacterized domain found frequently in the N-terminal section of ComEC homologues; pfam03772 is an uncharacterized domain ubiquitously present in ComEC homologues; smart00849, present in many ComEC homologues, may bind zinc ions as a cofactor (14); DZR stands for the double zinc ribbon domain most often involved in DNA binding in transcriptional regulators (38); PRTase stands for phosphoribosyltransferase, a domain often involved in nucleotide salvage pathways (37); MgCh-ChlI stands for magnesium chelatase subunit ChlI, an AAA+ ATPase that provides energy for insertion of $Mg^{2+}$ into protoporphyrin IX (40); RvuB is a domain found in some Holliday junction DNA helicases (41); MCM2/3/5 stands for maintenance of minichromosomes and functions in the replication of genomes (42).

DprA homologue contains a DprA domain 3 (DD3) (see Fig. S1), which is predicted to confer dsDNA binding capability to DprA proteins (13). Based on these analyses, we speculate that ComM is involved in a recombination step within the cytoplasm of recipient cells, and that its function is related to the DD3 (13, 18).

***comEC*, *comF*, and *comM* are required for RcGTA recipient capability.** The hypothesis that *comEC* (*rcc02362*), *comF* (*rcc00197*), and *comM* (*rcc00460*) are involved in RcGTA recipient capability was addressed by generating knockouts of these genes.

Null mutations of *comEC*, *comF*, or *comM* all resulted in an absolute loss in RcGTA recipient capability ($<10^{-6}$ of WT levels), and these mutations were complemented to WT levels by the native gene in *trans* (Fig. 2A). In all three mutant strains, the growth kinetics, RcGTA adsorption capability, UV sensitivity, and frequency of RecA-dependent integration of a suicide plasmid containing an insert homologous to a region on the chromosome were found to be identical to those of the WT strain (Fig. 2; also see Fig. S2 in the supplemental material). Therefore, the *R. capsulatus comEC*, *comF*, and *comM* homologues are essential for RcGTA recipient capability, but these genes are not needed for RcGTA binding to cells, RecA-mediated homologous recombination, growth, or UV damage-induced DNA repair, indicating that the defect(s) in these mutants is specific to RcGTA recipient capability at some stage after particle adsorption to cells. Because ComEC and ComF are essential for DNA transport through the CM in natural competence systems (14, 19, 36), we hypothesized that RcGTA-borne DNA enters the recipient cell cytoplasm via the ComEC/ComF machinery. A corollary of this hypothesis is that there would be a build-up of RcGTA-borne DNA in the periplasm of recipient cells lacking either the ComEC or ComF protein, and that this DNA could be detected in biochemical experiments.

**RcGTA-borne DNA build-up in the periplasmic fraction of Δ*comEC* and Δ*comF* mutants.** To track the relative amount of RcGTA-borne DNA in the periplasm and cytoplasm of recipient cells, a modified RcGTA transduction assay of a $Gm^r$ cartridge was used to monitor incoming DNA entry into the cell (see Materials and Methods).

DNA was purified from the periplasmic and cytoplasmic fractions after 3 and 24 h of incubation with RcGTA. A nonquantitative PCR of each fraction revealed that RcGTA-borne DNA was present in the periplasmic but not the cytoplasmic fraction of WT, Δ*comEC*, and Δ*comF* strains 3 h after exposure to RcGTA (Fig. 3A). However, after 24 h, the RcGTA-borne $Gm^r$ marker was detectable within the cytoplasm of only WT and not mutant cells (Fig. 3B). These data indicate that the *comEC* and *comF* genes are needed to import DNA from RcGTA to the cytoplasm, and that at some point between 3 and 24 h, the DNA is degraded in the periplasm of the Δ*comEC* and Δ*comF* strains. To more directly evaluate whether ComEC and ComF play a role in DNA transport through the CM, we quantified the amount of RcGTA-borne DNA in the periplasmic fraction at the 3-h time point; if there were a greater amount of RcGTA-borne DNA in the periplasm of Δ*comEC* and Δ*comF* mutants than in the WT strain, then ComEC and ComF would facilitate transport of DNA through the CM.

To quantify the residual periplasmic RcGTA-derived DNA at the 3-h time point, qPCR was performed using the $Gm^r$ cartridge as a target. Values obtained were normalized to the amounts of the *puhA* (photosynthetic reaction center) single-copy gene in the chromosomal fraction. It was found that the Δ*comEC* and Δ*comF* mutants contained 6.7-fold and 5.7-fold more incoming RcGTA DNA, respectively, in the periplasmic fraction than WT cells at this time point (Fig. 3C). These data indicate that the defect in RcGTA-borne gene acquisition in the Δ*comEC* and Δ*comF* mutants is due to a bottleneck in DNA transport from the periplasm to the cytoplasm.

**Prevalence of ComEC and ComF in GTA-containing organisms.** The RcGTA major structural gene cluster and close homologues appear limited to and vertically inherited in the alphaproteobacteria (5, 43). In addition to RcGTA, several other functional GTAs have been observed, such as in *Ruegeria mobilis*, *Ruegeria pomeroyi*, and *Roseovarious nubinhibens* (5, 44, 45). Furthermore, RcGTA-like full gene clusters, or partial/rearranged clusters, were present in most of the alphaproteobacterial genomes (43). Although genes encoding *comEC* and *comF* homologues are widespread, they are not ubiquitous in prokaryotes (14). We hypoth-

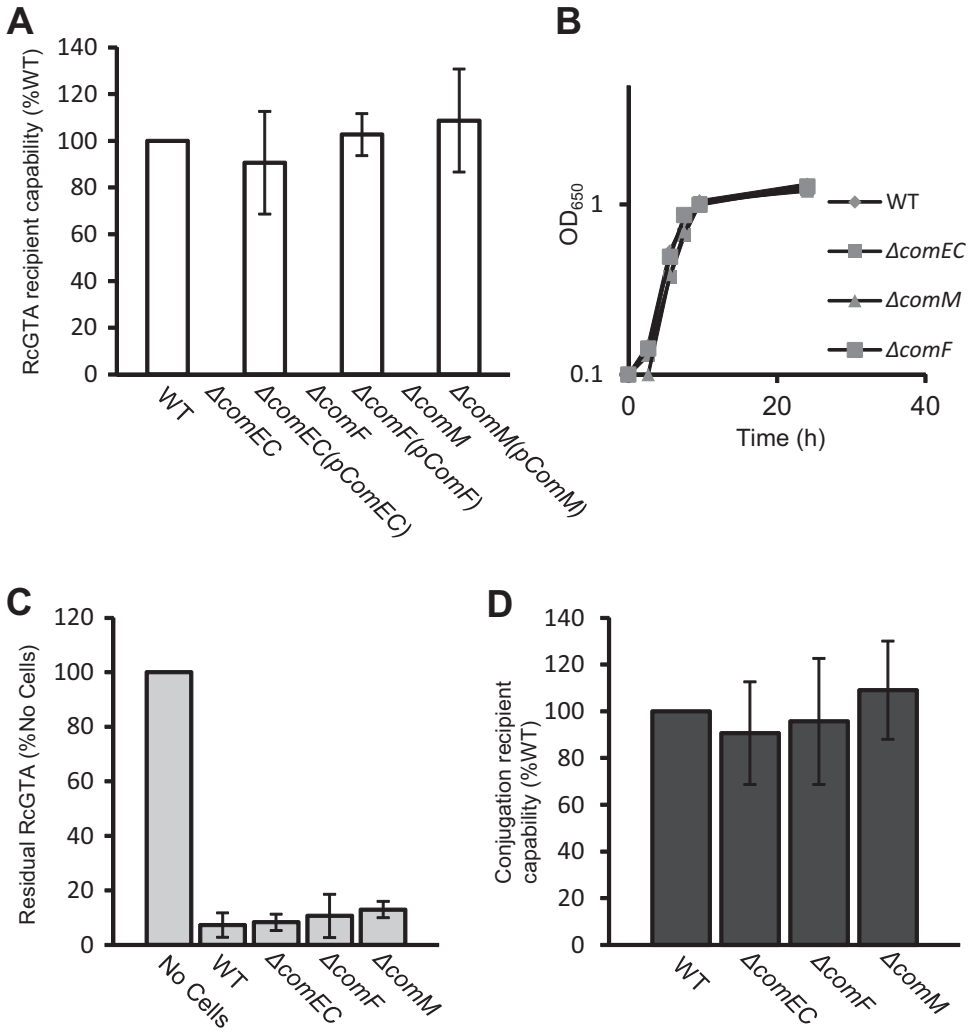


**FIG 2** (A) Relative RcGTA recipient capability of WT, Δ*comEC*, Δ*comF*, and Δ*comM* strains and the *trans*-complemented Δ*comEC*(pComEC), Δ*comF*(pComF), and Δ*comM*(pComM) strains. Error bars represent standard deviations from the means ($n = 3$). (B) Comparison of the growth rate of WT, Δ*comEC*, Δ*comF*, and Δ*comM* strains grown in RCV liquid medium aerobically with shaking at 200 rpm. Culture turbidity ($OD_{650}$) was measured for each strain at the indicated time points and plotted as a function of time. (C) Relative RcGTA adsorption capability of WT, Δ*comEC*, Δ*comF*, and Δ*comM* strains. Error bars represent the standard deviations from the means ($n = 3$). (D) Relative homologous recombination frequency of the suicide plasmid pZDJ1081 into the chromosome of WT, Δ*comEC*, Δ*comF*, and Δ*comM* strains, displayed as relative conjugation efficiency (%WT). Error bars represent the standard deviations from the means ($n = 3$).

esized that if *com* genes are important for GTA-mediated genetic exchange, homologues would be present in all previously identified GTA gene-containing genomes (43). Therefore, we performed BLASTP searches using the *R. capsulatus* genes as a query against a number of such alphaproteobacterial genomes, as well as unrelated bacteria that produce a non-RcGTA-homologous GTA. We observed that of 23 organisms that contain a functional GTA (either homologous to RcGTA or not) or those that contain full or partial/rearranged RcGTA-like gene clusters, all contain a ComEC homologue and a ComF homologue, except for *Sphingomonas alaskensis*, which contains only a ComEC homologue (summarized in Table S5 in the supplemental material).

**The RcGTA regulator CtrA is expressed throughout the population.** CtrA-dependent transcription of RcGTA genes is limited to <1% of a WT population (7, 46). Because CtrA is essential for *R. capsulatus* RcGTA recipient capability and the natural competence gene homologues *comEC*, *comF*, *comM*, and *dprA* are downregulated in a Δ*ctrA* mutant, we evaluated *ctrA* expression in single cells as an indicator of RcGTA recipient capability. For this purpose, an mCherry fluorescent protein coding sequence fused in frame with the *ctrA* start codon on plasmid pCtrAmCherry was conjugated into the WT strain B10. Cells were grown to the stationary phase and imaged by phase-contrast and fluorescence microscopy. It was found that *ctrA*, like the CtrA-induced competence gene *dprA*, is expressed in the vast majority of cells (Fig. 4A). Furthermore, a single peak of fluorescent cells was obtained when the culture was evaluated by flow cytometry (Fig. 4B).

## DISCUSSION

We provide genetic and biochemical evidence that ComEC/ComF natural competence-related machinery facilitates DNA carried within a phage-like RcGTA particle to enter the cytoplasm of recipient cells, and that the ComM protein of unknown function is involved in RcGTA recipient capability.

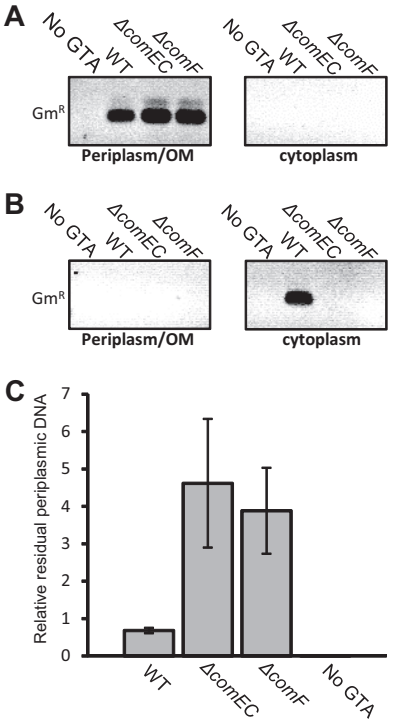


**FIG 3** Tracking of RcGTA-borne DNA levels in the periplasm and cytoplasm of WT, Δ*comEC*, and Δ*comF* strains. (A) PCR products obtained from cell fractions after incubation with RcGTA for 3 h. (B) PCR products obtained from cell fractions after incubation with RcGTA for 24 h. (C) qPCR values, displayed as ratios of periplasmic RcGTA-borne DNA to chromosomal *puhA* levels. Error bars represent the standard deviations from the means ($n \geq 3$). Statistical analysis was done by one-way ANOVA, with the results given in Table S6 in the supplemental material.

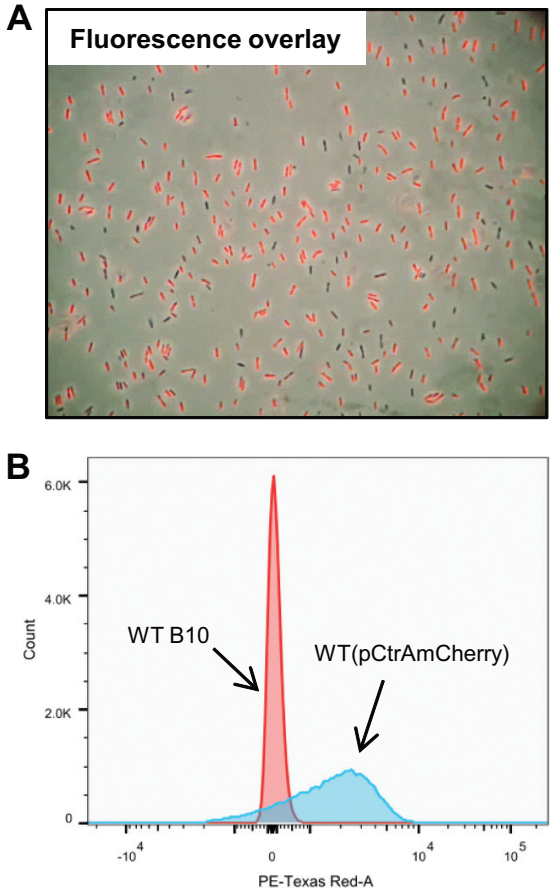


**FIG 4** (A) Fluorescence image where WT(pCtrAmCherry) cells were excited by 561-nm light (fluorescence emission, 610 nm) overlain on a light microscopy image of the same cells. (B) Flow cytometry graphical representation of WT cells containing the plasmid pCtrAmCherry, on which the mCherry fluorescent protein gene is driven by the *ctrA* promoter, compared to WT cells lacking the plasmid. The horizontal axis represents arbitrary fluorescence units, and the vertical axis gives cell counts (the total number of cells in each sample, indicated by the integrated area under each curve, was 50,000). PE, phycoerythrin.

The RcGTA morphologically resembles a small, noncontractile tailed phage (8), and based on the data presented in this report, we suggest that RcGTA uses a mode of DNA entry into the cell different from that of phage model systems.

During infection by the contractile long-tailed phage T4, receptor binding triggers a conformational change resulting in the contraction of the tail sheath, causing the tail tube to puncture the outer membrane of the recipient cell. The tail tube then penetrates the peptidoglycan (PG), which is cleaved by the lysozyme domain on the tail tube (gp5), the tail tube crosses the cytoplasmic membrane, and subsequently it delivers DNA into the cytoplasm (47, 48).

The noncontractile long-tailed phage λ differs from T4. An initial binding to the LamB receptor (49) triggers a conformational change in the tail, bringing it into contact with the cell surface. This triggers release of the DNA, which is thought to be accompanied by movement of the tape measure protein (TMP) and central tail fiber protein from the tail into the cell envelope, forming a channel for DNA entry into the periplasm (50) and subsequently into the cytoplasm in a process involving the mannose permease complex, although the mechanism is not understood (50–52). The process of breaching the peptidoglycan also is unclear.

Infection by short-tailed phages, such as T7 or p22, begins with initial receptor binding via tail fibers. Often, interaction with a secondary receptor (such as an integral OM protein) also is required. Proteins present in the head then are injected into the periplasm via the tail prior to DNA entry, and these proteins may degrade the peptidoglycan. Subsequently, the short tail is lengthened, or a translocation tube is formed by injected proteins to form a channel into the cytoplasm, allowing DNA delivery (53, 54). In addition, it was recently shown that the apparently tail-less phage ϕX174 forms a tail-like structure during infection to span the periplasmic space for DNA transport (55).

Our data indicate that there is an accumulation of RcGTA-borne DNA in the periplasm of Δ*comEC* and Δ*comF* mutants. One possible explanation for the bottleneck is that the ComEC DNA transporter serves as a CM receptor for a lengthening tail or a TMP, analogous to the poorly understood function of ManY for phage λ (50, 51, 56). In this scenario, the CM receptor is not required for DNA ejection from the particle that has docked on an OM receptor but is required for injection into the cytoplasm, so the DNA comes to reside in the periplasm. Alternatively, RcGTA may inject DNA into the periplasm of WT cells and the ComEC/ComF proteins transport the DNA through the CM, as in natural

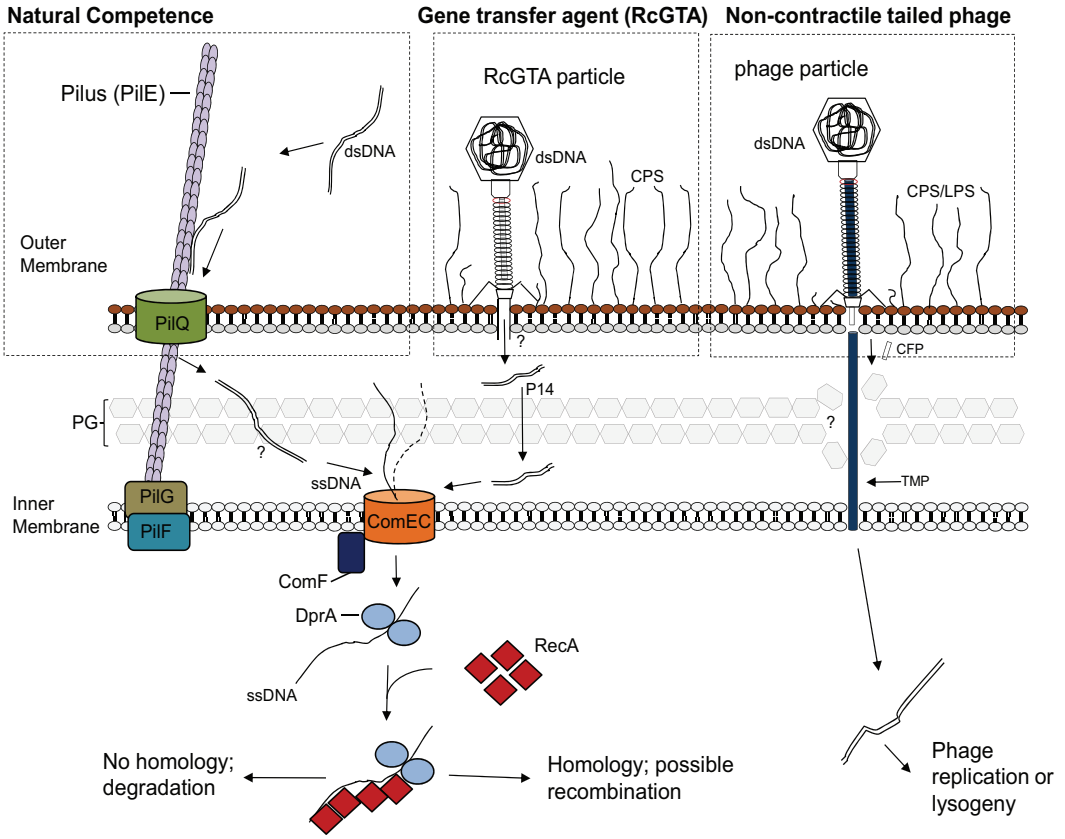


**FIG 5** Schematic diagram comparing an overview of natural transformation systems in Gram-negative bacteria to the proposed RcGTA recipient capability pathway and a noncontractile tailed phage pathway. The conserved steps in natural transformation are shown on the left. DNA is taken up by a transformation-dedicated pilus (Tfb), which binds dsDNA and brings it into the periplasm, where it is processed into ssDNA by an unknown process. DNA then enters the cytoplasm via the ComEC inner membrane protein in a pathway involving ComF. Upon cytoplasmic internalization, ssDNA is bound by DprA, which functions to recruit the RecA recombinase to polymerize onto ssDNA, promoting a homology search and subsequent recombination into the chromosome (14). The proposed model of RcGTA, shown in the center, involves the same steps for DNA import and recombination into the chromosome as those in natural transformation. It is unknown how RcGTA-injected DNA traverses the periplasm or whether DNA enters the cell as ssDNA or dsDNA. Passage through the peptidoglycan (PG) likely is facilitated by the RcGTA protein p14, which degrades PG (46). Shown on the right is a model of noncontractile tailed phage infection, with the tape measure protein (TMP) used to traverse the outer membrane, periplasm, and cytoplasmic membrane (50). CFP, central tail fiber protein; LPS, lipopolysaccharide; CPS, capsular polysaccharide. (The natural transformation pathway image was adapted from reference 14 with permission of the publisher.)

transformation (14). Although our data do not definitively differentiate between these two possibilities, we favor the latter explanation for several reasons.

One reason is the fact that both *comEC* and *comF* expression are controlled by CtrA as part of a set of natural transformation genes, which also includes the RcGTA-borne DNA-specific cytoplasmic recombination mediator DprA (13). This indicates that all of these genes are part of a regulated system of DNA uptake and recombination, which contrasts with the use of ManY (which has a function unrelated to DNA transport) by phage λ (50). Another reason is the need for ComF in DNA passage through the CM. Because the bulk of ComF is predicted to reside in the cytoplasm, it appears unlikely to be used as an IM receptor. Lastly, we note that the RcGTA predicted TMP protein is only 219 amino acids in length, which is much shorter than typical noncontractile tailed phage TMPs (>600 amino acids) that are thought to bridge the periplasmic space (50). A model is shown in Fig. 5.

Although the phenotype of *comEC* and *comF* null mutants has been documented in several studies of natural transformation systems (19, 36), the role of *comM* homologues has not. Here, we establish that the *R. capsulatus comM* homologue is essential for detectable RcGTA recipient capability. This is in contrast to transformation of *H. influenzae*, which was reduced by only ~6-fold (19) in a *comM* mutant, although the reason for this is not clear. One interesting observation we made was that genes encoding ComM homologues appear to be absent from genomes containing DprA homologues that lack a DD3, including S. *pneumoniae* and *B. subtilis*, in which DprA proteins have been extensively studied and bind ssDNA but not dsDNA. Because of the cooccurrence of ComM homologues and DprA proteins that contain a DD3, we speculate that ComM and DprA function in the same pathway. Because DprA is directly involved in recombination in natural transformation and ComM is predicted to be cytoplasmic and contains predicted domains similar to those of helicases, we speculate that ComM is involved in a recombination step in the cytoplasm of *R. capsulatus* cells and possibly in natural transformation systems as well.

CtrA is a widely conserved regulator among the alphaproteobacteria. In the prototypic example of *Caulobacter crescentus*, CtrA is a regulator of flagellar motility, the cell cycle, and asymmetric cell division, and it is essential for viability (57–59). In *R. capsulatus*, *ctrA* is not essential for viability but was found to control a

regulon partially overlapping that of *C. crescentus*, including flagellum synthesis (60, 61). Although *R. capsulatus* CtrA is essential for both RcGTA production and recipient capability (13, 60, 62), the RcGTA promoter is activated in <1% of WT cells in the stationary phase (7, 46). In contrast, the essential recipient capability factor (*dprA*) promoter is expressed in essentially all cells in a population (13), as we found here for *ctrA* itself (Fig. 4). The mechanism for restricting RcGTA production to a subset of CtrA-induced cells remains to be determined.

Because RcGTA genetic exchange requires homologous recombination within the same species, coregulation of RcGTA production and recipient capability may be explained in terms of natural selection. Although RcGTA production requires lysis of the producing cell, only a small fraction of a population expresses these genes (7). Because the cost of producing RcGTA (i.e., death) is high, having the surrounding population primed as RcGTA recipients by transcription of the *com* genes would provide a selective advantage to such cells, because they could take up the DNA packaged by the producing cells, perhaps facilitating spread of beneficial mutations HR-mediated DNA repair during growth under mutation-prone conditions.

We suggest that RcGTA represents a mechanism of horizontal gene transfer that combines aspects of transduction and natural transformation, resulting in a novel genetic exchange mechanism that provides recipients with new genes or alleles. In a natural aquatic environment, where RcGTA-producing *Rhodobacteraceae* are prevalent (63), the encapsidation of DNA within RcGTA would protect the DNA from degradation by nucleases until the particle encountered a cell that had been rendered capable of acquisition of RcGTA-borne genes, via *com* gene induction, regulated by the same systems that induced the production of RcGTA particles.

## ACKNOWLEDGMENTS

We thank L. Eltis for access to qPCR components and H. Otani for technical assistance. We thank the Canadian Institutes of Health Research for an Operating Grant (93779 to J.T.B.) and the University of British Columbia for a postgraduate scholarship (to C.A.B.).

C.A.B. designed research, performed experiments, analyzed data, and wrote the manuscript; H.D. performed fluorescence microscopy analysis, analyzed data, and wrote the manuscript; J.A.J. generated the Δ*comEC* mutant and edited the manuscript; J.T.B. designed research, analyzed data, and wrote the manuscript.

## REFERENCES

1. **Griffith F.** 1928. The significance of *Pneumococcal* types. J Hyg **27:**113–159. http://dx.doi.org/10.1017/S0022172400031879.
2. **Lederberg J, Tatum EL.** 1946. Gene recombination in *Escherichia coli*. Nature **158:**558.
3. **Zinder ND, Lederberg J.** 1952. Genetic exchange in *Salmonella*. J Bacteriol **64:**679–699.
4. **Marrs B.** 1974. Genetic recombination in *Rhodopseudomonas capsulata*. Proc Natl Acad Sci U S A **71:**971–973. http://dx.doi.org/10.1073/pnas.71.3.971.
5. **Lang AS, Zhaxybayeva O, Beatty JT.** 2012. Gene transfer agents: phage-like elements of genetic exchange. Nat Rev Microbiol **10:**472–482.
6. **Lang AS, Beatty JT.** 2001. The gene transfer agent of *Rhodobacter capsulatus* and "constitutive transduction" in prokaryotes. Arch Microbiol **175:**241–249. http://dx.doi.org/10.1007/s002030100260.
7. **Hynes AP, Mercer RG, Watton DE, Buckley CB, Lang AS.** 2012. DNA packaging bias and differential expression of gene transfer agent genes within a population during production and release of the *Rhodobacter capsulatus* gene transfer agent, RcGTA. Mol Microbiol **85:**314–325. http://dx.doi.org/10.1111/j.1365-2958.2012.08113.x.
8. **Yen HC, Hu NT, Marrs BL.** 1979. Characterization of the gene transfer agent made by an overproducer mutant of *Rhodopseudomonas capsulata*. J Mol Biol **131:**157–168. http://dx.doi.org/10.1016/0022-2836(79)90071-8.
9. **Westbye AB, Leung MM, Florizone SM, Taylor TA, Johnson JA, Fogg PC, Beatty JT.** 2013. Phosphate concentration and the putative sensor kinase protein CckA modulate cell lysis and release of the *Rhodobacter capsulatus* gene transfer agent. J Bacteriol **195:**5025–5040. http://dx.doi.org/10.1128/JB.00669-13.
10. **Schaefer AL, Taylor TA, Beatty JT, Greenberg EP.** 2002. Long-chain acyl-homoserine lactone quorum-sensing regulation of *Rhodobacter capsulatus* gene transfer agent production. J Bacteriol **184:**6515–6521. http://dx.doi.org/10.1128/JB.184.23.6515-6521.2002.
11. **Lang AS, Beatty JT.** 2002. A bacterial signal transduction system controls genetic exchange and motility. J Bacteriol **184:**913–918. http://dx.doi.org/10.1128/jb.184.4.913-918.2002.
12. **Leung MM, Brimacombe CA, Spiegelman GB, Beatty JT.** 2012. The GtaR protein negatively regulates transcription of the *gtaRI* operon and modulates gene transfer agent (RcGTA) expression in *Rhodobacter capsulatus*. Mol Microbiol **83:**759–774. http://dx.doi.org/10.1111/j.1365-2958.2011.07963.x.
13. **Brimacombe CA, Ding H, Beatty JT.** 2014. *Rhodobacter capsulatus* DprA is essential for RecA-mediated gene transfer agent (RcGTA) recipient capability regulated by quorum-sensing and the CtrA response regulator. Mol Microbiol **92:**1260–1278. http://dx.doi.org/10.1111/mmi.12628.
14. **Johnston C, Martin B, Fichant G, Polard P, Claverys JP.** 2014. Bacterial transformation: distribution, shared mechanisms and divergent control. Nat Rev Microbiol **12:**181–196. http://dx.doi.org/10.1038/nrmicro3199.
15. **Mell JC, Hall IM, Redfield RJ.** 2012. Defining the DNA uptake specificity of naturally competent *Haemophilus influenzae* cells. Nucleic Acids Res **40:**8536–8549. http://dx.doi.org/10.1093/nar/gks640.
16. **Draskovic I, Dubnau D.** 2005. Biogenesis of a putative channel protein, ComEC, required for DNA uptake: membrane topology, oligomerization and formation of disulphide bonds. Mol Microbiol **55:**881–896.
17. **Mortier-Barriere I, Velten M, Dupaigne P, Mirouze N, Pietrement O, McGovern S, Fichant G, Martin B, Noirot P, Le Cam E, Polard P, Claverys JP.** 2007. A key presynaptic role in transformation for a widespread bacterial protein: DprA conveys incoming ssDNA to RecA. Cell **130:**824–836. http://dx.doi.org/10.1016/j.cell.2007.07.038.
18. **Yadav T, Carrasco B, Hejna J, Suzuki Y, Takeyasu K, Alonso JC.** 2013. *Bacillus subtilis* DprA recruits RecA onto single-stranded DNA and mediates annealing of complementary strands coated by SsbB and SsbA. J Biol Chem **288:**22437–22450. http://dx.doi.org/10.1074/jbc.M113.478347.
19. **Sinha S, Mell JC, Redfield RJ.** 2012. Seventeen Sxy-dependent cyclic AMP receptor protein site-regulated genes are needed for natural transformation in *Haemophilus influenzae*. J Bacteriol **194:**5245–5254. http://dx.doi.org/10.1128/JB.00671-12.
20. **Fogg PC, Colloms S, Rosser S, Stark M, Smith MC.** 2014. New applications for phage integrases. J Mol Biol **426:**2703–2716. http://dx.doi.org/10.1016/j.jmb.2014.05.014.
21. **Groth AC, Calos MP.** 2004. Phage integrases: biology and applications. J Mol Biol **335:**667–678. http://dx.doi.org/10.1016/j.jmb.2003.09.082.
22. **Simon R, Priefer U, Puhler A.** 1983. A broad host range mobilization system for in vivo genetic engineering: transposon mutagenesis in gram negative bacteria. Nat Biotech **1:**784–791. http://dx.doi.org/10.1038/nbt1183-784.
23. **Taylor DP, Cohen SN, Clark WG, Marrs BL.** 1983. Alignment of genetic and restriction maps of the photosynthesis region of the *Rhodopseudomonas capsulata* chromosome by a conjugation-mediated marker rescue technique. J Bacteriol **154:**580–590.
24. **Sambrook J, Fritsch EF, Maniatis T.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Plainview, NY.
25. **Beatty JT, Gest H.** 1981. Biosynthetic and bioenergetic functions of citric acid cycle reactions in *Rhodopseudomonas capsulata*. J Bacteriol **148:**584–593.
26. **Wall JD, Weaver PF, Gest H.** 1975. Gene transfer agents, bacteriophages, and bacteriocins of *Rhodopseudomonas capsulata*. Arch Microbiol **105:**217–224. http://dx.doi.org/10.1007/BF00447140.
27. **Brimacombe CA, Stevens A, Jun D, Mercer R, Lang AS, Beatty JT.** 2013. Quorum-sensing regulation of a capsular polysaccharide receptor for the *Rhodobacter capsulatus* gene transfer agent (RcGTA). Mol Microbiol **87:**802–817. http://dx.doi.org/10.1111/mmi.12132.
28. **Barany F.** 1985. Single-stranded hexameric linkers: a system for in-phase

insertion mutagenesis and protein engineering. Gene **37:**111–123. http://dx.doi.org/10.1016/0378-1119(85)90263-X.
29. **Marx CJ, Lidstrom ME.** 2001. Development of improved versatile broad-host-range vectors for use in methylotrophs and other Gram-negative bacteria. Microbiology **147:**2065–2075.
30. **Hancock RE.** 1984. Alterations in outer membrane permeability. Annu Rev Microbiol **38:**237–264. http://dx.doi.org/10.1146/annurev.mi.38.100184.001321.
31. **Dereeper A, Audic S, Claverie JM, Blanc G.** 2010. BLAST-EXPLORER helps you building datasets for phylogenetic analysis. BMC Evol Biol **10:**8. http://dx.doi.org/10.1186/1471-2148-10-8.
32. **McWilliam H, Li W, Uludag M, Squizzato S, Park YM, Buso N, Cowley AP, Lopez R.** 2013. Analysis tool web services from the EMBL-EBI. Nucleic Acids Res **41:**W597–W600. http://dx.doi.org/10.1093/nar/gkt376.
33. **Edgar RC.** 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res **32:**1792–1797. http://dx.doi.org/10.1093/nar/gkh340.
34. **Saitou N, Nei M.** 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol **4:**406–425.
35. **Finn RD, Bateman A, Clements J, Coggill P, Eberhardt RY, Eddy SR, Heger A, Hetherington K, Holm L, Mistry J, Sonnhammer EL, Tate J, Punta M.** 2014. Pfam: the protein families database. Nucleic Acids Res **42:**D222–D230. http://dx.doi.org/10.1093/nar/gkt1223.
36. **Seitz P, Blokesch M.** 2013. DNA-uptake machinery of naturally competent *Vibrio cholerae*. Proc Natl Acad Sci U S A **110:**17987–17992. http://dx.doi.org/10.1073/pnas.1315647110.
37. **Murray AW.** 1971. The biological significance of purine salvage. Annu Rev Biochem **40:**811–826. http://dx.doi.org/10.1146/annurev.bi.40.070171.004115.
38. **Klug A, Rhodes D.** 1987. Zinc fingers: a novel protein fold for nucleic acid recognition. Cold Spring Harbor Symp Quant Biol **52:**473–482. http://dx.doi.org/10.1101/SQB.1987.052.01.054.
39. **Gwinn ML, Ramanathan R, Smith HO, Tomb JF.** 1998. A new transformation-deficient mutant of *Haemophilus influenzae* Rd with normal DNA uptake. J Bacteriol **180:**746–748.
40. **Reid JD, Siebert CA, Bullough PA, Hunter CN.** 2003. The ATPase activity of the ChlI subunit of magnesium chelatase and formation of a heptameric AAA+ ring. Biochemistry **42:**6912–6920. http://dx.doi.org/10.1021/bi034082q.
41. **Yamada K, Miyata T, Tsuchiya D, Oyama T, Fujiwara Y, Ohnishi T, Iwasaki H, Shinagawa H, Ariyoshi M, Mayanagi K, Morikawa K.** 2002. Crystal structure of the RuvA-RuvB complex: a structural basis for the Holliday junction migrating motor machinery. Mol Cell **10:**671–681. http://dx.doi.org/10.1016/S1097-2765(02)00641-X.
42. **Maiorano D, Lutzmann M, Mechali M.** 2006. MCM proteins and DNA replication. Curr Opin Cell Biol **18:**130–136. http://dx.doi.org/10.1016/j.ceb.2006.02.006.
43. **Lang AS, Beatty JT.** 2007. Importance of widespread gene transfer agent genes in alpha-proteobacteria. Trends Microbiol **15:**54–62. http://dx.doi.org/10.1016/j.tim.2006.12.001.
44. **Biers EJ, Wang K, Pennington C, Belas R, Chen F, Moran MA.** 2008. Occurrence and expression of gene transfer agent genes in marine bacterioplankton. Appl Environ Microbiol **74:**2933–2939. http://dx.doi.org/10.1128/AEM.02129-07.
45. **McDaniel LD, Young E, Delaney J, Ruhnau F, Ritchie KB, Paul JH.** 2010. High frequency of horizontal gene transfer in the oceans. Science **330:**50. http://dx.doi.org/10.1126/science.1192243.
46. **Fogg PCM, Westbye AB, Beatty JT.** 2012. One for all or all for one: heterogeneous expression and host cell lysis are key to gene transfer agent activity in *Rhodobacter capsulatus*. PLoS One **7:**e43772. http://dx.doi.org/10.1371/journal.pone.0043772.
47. **Rossmann MG, Mesyanzhinov VV, Arisaka F, Leiman PG.** 2004. The bacteriophage T4 DNA injection machine. Curr Opin Struct Biol **14:**171–180. http://dx.doi.org/10.1016/j.sbi.2004.02.001.
48. **Leiman PG, Shneider MM.** 2012. Contractile tail machines of bacteriophages. Adv Exp Med Biol **726:**93–114.
49. **Schwartz M.** 1975. Reversible interaction between coliphage lambda and its receptor protein. J Mol Biol **99:**185–201. http://dx.doi.org/10.1016/S0022-2836(75)80167-7.
50. **Davidson AR, Cardarelli L, Pell LG, Radford DR, Maxwell KL.** 2012. Long noncontractile tail machines of bacteriophages. Adv Exp Med Biol **726:**115–142.
51. **Williams N, Fox DK, Shea C, Roseman S.** 1986. Pel, the protein that permits lambda DNA penetration of *Escherichia coli*, is encoded by a gene in *ptsM* and is required for mannose utilization by the phosphotransferase system. Proc Natl Acad Sci U S A **83:**8934–8938. http://dx.doi.org/10.1073/pnas.83.23.8934.
52. **Scandella D, Arber W.** 1976. Phage lambda DNA injection into *Escherichia coli pel-* mutants is restored by mutations in phage genes V or H. Virology **69:**206–215. http://dx.doi.org/10.1016/0042-6822(76)90207-5.
53. **Casjens SR, Molineux IJ.** 2012. Short noncontractile tail machines: adsorption and DNA delivery by podoviruses. Adv Exp Med Biol **726:**143–179.
54. **Hu B, Margolin W, Molineux IJ, Liu J.** 2013. The bacteriophage t7 virion undergoes extensive structural remodeling during infection. Science **339:**576–579. http://dx.doi.org/10.1126/science.1231887.
55. **Sun L, Young LN, Zhang X, Boudko SP, Fokine A, Zbornik E, Roznowski AP, Molineux IJ, Rossmann MG, Fane BA.** 2014. Icosahedral bacteriophage PhiX174 forms a tail for DNA transport during infection. Nature **505:**432–435.
56. **Boulanger P, Jacquot P, Plancon L, Chami M, Engel A, Parquet C, Herbeuval C, Letellier L.** 2008. Phage T5 straight tail fiber is a multifunctional protein acting as a tape measure and carrying fusogenic and muralytic activities. J Biol Chem **283:**13556–13564. http://dx.doi.org/10.1074/jbc.M800052200.
57. **Quon KC, Marczynski GT, Shapiro L.** 1996. Cell cycle control by an essential bacterial two-component signal transduction protein. Cell **84:**83–93. http://dx.doi.org/10.1016/S0092-8674(00)80995-2.
58. **Wu J, Ohta N, Newton A.** 1998. An essential, multicomponent signal transduction pathway required for cell cycle regulation in *Caulobacter*. Proc Natl Acad Sci U S A **95:**1443–1448. http://dx.doi.org/10.1073/pnas.95.4.1443.
59. **Reisenauer A, Quon K, Shapiro L.** 1999. The CtrA response regulator mediates temporal control of gene expression during the *Caulobacter* cell cycle. J Bacteriol **181:**2430–2439.
60. **Lang AS, Beatty JT.** 2000. Genetic analysis of a bacterial genetic exchange element: the gene transfer agent of *Rhodobacter capsulatus*. Proc Natl Acad Sci U S A **97:**859–864. http://dx.doi.org/10.1073/pnas.97.2.859.
61. **Mercer RG, Callister SJ, Lipton MS, Pasa-Tolic L, Strnad H, Paces V, Beatty JT, Lang AS.** 2010. Loss of the response regulator CtrA causes pleiotropic effects on gene expression but does not affect growth phase regulation in *Rhodobacter capsulatus*. J Bacteriol **192:**2701–2710. http://dx.doi.org/10.1128/JB.00160-10.
62. **Mercer RG, Quinlan M, Rose AR, Noll S, Beatty JT, Lang AS.** 2012. Regulatory systems controlling motility and gene transfer agent production and release in *Rhodobacter capsulatus*. FEMS Microbiol Lett **331:**53–62. http://dx.doi.org/10.1111/j.1574-6968.2012.02553.x.
63. **Yu F, MacLeod DM, Rivkin RB, Chen F, Buchan A, Lang AS.** 2010. High diversity of *Rhodobacterales* in the subarctic north Atlantic Ocean and gene transfer agent protein expression in isolated strains. Aquatic Microb Ecol **59:**283–293. http://dx.doi.org/10.3354/ame01398.