

A Bacterial Signal Transduction System Controls Genetic Exchange and Motility

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The bacterium *Rhodobacter capsulatus* is capable of an unusual form of genetic exchange, mediated by a transducing bacteriophage-like particle called the gene transfer agent (GTA). GTA production by *R. capsulatus* is controlled at the level of transcription by a cellular two-component signal transduction system that includes a putative histidine kinase (CckA) and response regulator (CtrA). We found that, in addition to regulating genetic exchange by *R. capsulatus*, this signal transduction system controls motility. As with the regulation of GTA production, the control of motility by CckA and CtrA occurs through modulation of gene transcription. Disruptions of the *cckA* and *ctrA* genes resulted in a loss of class II, class III, and class IV flagellar gene transcripts, suggesting that *cckA* and *ctrA* function in motility as class I flagellar genes. We also found that, analogous to the GTA genes, transcription of *R. capsulatus* flagellar genes appears to be growth phase dependent: class II flagellar gene transcripts are maximal in the mid-log phase of the culture growth cycle, whereas class III gene transcripts are maximal in the late-log phase of growth. We speculate that coordinate regulation of motility and GTA-mediated genetic exchange in *R. capsulatus* exists because these two processes are complementary mechanisms for cells to cope with unfavorable conditions in natural environments.

Bacterial viruses (bacteriophage or phage) mediate genetic exchange (transduction), and this is an important mechanism by which genes are transferred between bacteria (17). An unusual genetic exchange element called the gene transfer agent (GTA) was discovered in the bacterium *Rhodobacter capsulatus* (16). The morphology of GTA is that of a tiny tailed phage (29), and it acts similarly to a generalized transducing phage, but GTA differs in part because every particle is capable of transduction. Each GTA particle contains ca. 4 kb of randomly packaged genomic DNA that may be injected into an *R. capsulatus* recipient, where allelic recombination may occur (24, 29). The capacity of a GTA particle is insufficient for transfer of the GTA structural genes because the GTA structural genes are organized in a ca. 15-kb cluster (12, 13). These GTA genes are maximally transcribed in the stationary phase of growth of laboratory cultures (13), which is when GTA is produced in the greatest quantities (25).

Two cellular genes, *ctrA* and *cckA*, positively regulate GTA production (13). These genes encode two-component signal transduction protein homologues; *ctrA* is predicted to encode a response regulator protein, and *cckA* is predicted to encode a sensor kinase protein. When the *ctrA* gene is mutated, GTA structural gene transcription becomes undetectable (13). Therefore, stationary-phase transcription of the GTA structural genes, and thus production of GTA particles that mediate genetic exchange between *R. capsulatus* cells, is regulated by a cellular two-component signaling system.

Many bacteria, including *R. capsulatus*, are motile by the use of flagella. The flagellum is a complicated structure that re-

quires a vast amount of protein and expression of more than 40 genes, and it consumes a large amount of energy for rotation (4). The assembly of the flagellum is under complex regulation, and the expression of flagellar genes is often controlled by signal transduction pathways and alternative RNA polymerase sigma (σ) factors (9, 15, 20). Flagellar genes can be divided into four classes (I to IV) that reflect their relative temporal order of expression. The products of each gene class are required for expression of the subsequent gene class, and so this order of expression reflects the required order of the proteins for assembly of the flagellum (20).

The *R. capsulatus ctrA* gene is located 3' of an open reading frame whose predicted product has high sequence similarity to a protein encoded in a *Caulobacter crescentus* flagellar gene cluster (13). This gene organization and the fact that CtrA is required for expression of flagellar genes in *C. crescentus* (19) led us to hypothesize that CtrA (and hence CckA) might regulate motility in *R. capsulatus*. Here we show that an *R. capsulatus ctrA* mutant is nonmotile, whereas a *cckA* mutant shows reduced motility, and that these defects in motility are due to reductions in the amounts of flagellar gene transcripts. Thus, in addition to a function in regulating genetic exchange by the activation of transcription of GTA structural genes, *R. capsulatus cckA* and *ctrA* are class I flagellar genes.

MATERIALS AND METHODS

Bacterial strains, growth conditions, and plasmids. The *Escherichia coli* strains used for subcloning and to conjugate plasmids into *R. capsulatus* are listed in Table 1. The *E. coli* strains were grown in Luria-Bertani medium (21) supplemented with the appropriate antibiotics at the following concentrations: ampicillin, 200 $\mu\text{g/ml}$; kanamycin sulfate, 50 $\mu\text{g/ml}$; and gentamicin sulfate, 8 $\mu\text{g/ml}$.

The *R. capsulatus* strains (Table 1) were grown aerobically in RCV minimal medium (3) supplemented with the appropriate antibiotics (kanamycin sulfate, 10 $\mu\text{g/ml}$; gentamicin sulfate, 3 $\mu\text{g/ml}$) or photosynthetically (anaerobically) in YPS complex medium (28). A Klett-Summerson photometer (red filter no. 66) was used to measure light scattering to monitor the turbidity of cultures.

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TABLE 1. Strains and plasmids

| Strain or plasmid | Relevant properties | Source or reference |
|----------------------|--|------------------------|
| Strains | | |
| <i>E. coli</i> | | |
| DH5 α | Subcloning strain | GIBCO-BRL ^a |
| DH10B | Subcloning strain | GIBCO-BRL |
| RB404 | Subcloning strain, <i>dam</i> mutant | 5 |
| S17-1 | Plasmid-mobilizing strain | 23 |
| C600(pDPT51) | Plasmid-mobilizing strain | 26 |
| <i>R. capsulatus</i> | | |
| B10 | Wild type | 16 |
| BCKF | B10 with <i>ctrA</i> ::KIXX disruption | This work |
| BKKR | B10 with <i>cckA</i> ::KIXX disruption | This work |
| Y262 | GTA overproducer | 29 |
| Plasmids | | |
| pUC13 | Subcloning vector | 27 |
| pUC13Hx | Subcloning vector, pUC13 with destroyed <i>Hind</i> III site | This work |
| pUC4KIXX | Source of KIXX cartridge | 2 |
| pCTRA | <i>R. capsulatus ctrA</i> expression plasmid | 13 |
| pHxCES | pUC13Hx containing <i>ctrA</i> gene | This work |
| pCHBKIXF2 | pHxCES with KIXX disruption in <i>ctrA</i> gene | This work |
| pCHP1 | pUC13 containing part of <i>cckA</i> gene | This work |
| pBgKR2 | pCHP1 with KIXX disruption in <i>cckA</i> gene | This work |

^a Rockville, Md.

The plasmids used are also listed in Table 1.

Motility tests. Motility was evaluated by stabbing YPS soft agar (0.4%) in test tubes and incubating the samples under photosynthetic conditions at 35°C for 3 days.

Mutant construction. Gene disruptions were made by ligating the *neo* gene-containing *Sma*I fragment (KIXX cartridge) from the plasmid pUC4KIXX (2) into the cloned gene to be disrupted. These mutations were made in plasmids that were subsequently transferred into the *R. capsulatus* GTA overproducer strain Y262 by conjugation from *E. coli* C600(pDPT51) (26). The mutations were then transferred to *R. capsulatus* strain B10 chromosome by GTA transduction as described previously (22); the genotypes of the resulting mutant strains are represented in Fig. 1.

Strain BCKF was constructed from strain B10 by replacing the *ctrA* gene with a KIXX-disrupted version. The *ctrA* coding region between nucleotides 124 and 672 was deleted by digestion of plasmid pHxCES with *Hind*III and *Bcl*I, the ends were made blunt with Klenow enzyme, and the KIXX cartridge was inserted into this site to generate pCHBKIXF2.

Strain BKKR was constructed from strain B10 by replacement of the *cckA* gene (on plasmid pCHP1) with a sequence in which the KIXX cartridge had been placed in the *cckA* gene at the blunted (Klenow) *Bgl*II site, 606 nucleotides from the 3' end of the coding sequence (yielding plasmid pBgKR2).

RNA analysis. Photosynthetically grown *R. capsulatus* cells were harvested at the desired point in the growth phase, as determined by culture turbidity. The exponential phase of photosynthetic growth occurred from ca. 25 to 350 Klett units (KU), with the mid-log phase defined as 180 to 200 KU, the late-log phase defined as 320 to 340 KU, and the early stationary phase defined as 1 h after the culture stopped increasing in turbidity (usually at ca. 350 KU). For *R. capsulatus*, 100 KU represents ca. 4×10^8 cells/ml. RNA was isolated by using the RNeasy kit (Qiagen). RNA electrophoresis and blotting were done as described previously (14). DNA probes were [α -³²P]dCTP labeled with the Rediprime kit (Amersham). Blots were incubated for 2 h at 42°C in hybridization buffer (50% formamide, 10% dextran sulfate, 1.0 M NaCl, 50 mM Tris-Cl [pH 7.5], 0.2% bovine serum albumin, 0.2% Ficoll, 0.1% sodium pyrophosphate, 1% sodium dodecyl sulfate [SDS], 0.2% polyvinylpyrrolidone, 0.1 mg of denatured sheared salmon sperm DNA/ml) before the probe (ca. 25 ng of labeled DNA) was added, and hybridizations were done overnight. Hybridized blots were washed as follows: two 5-min washes in 2 \times SSC (1 \times SSC is 0.15 M NaCl plus 0.015 M sodium citrate) (21) at room temperature, two 15-min washes in 2 \times SSC-1% SDS at 60°C, and two 15-min washes in 0.1 \times SSC at room temperature. Blots were then

placed on X-ray film in the presence of an intensifying screen at -80°C for various lengths of time. Blots were stripped before reprobing according to the manufacturer's (ICN) recommendations.

The DNA fragments used as probes were generated by PCR with *R. capsulatus* chromosomal DNA and the appropriate primer pair. Primers 3638F (5'-GATC TTCCAGCCGACCATCC-3') and 3638R (5'-ATGCCTGCACCACACCGAC-3') were used to generate the ca. 2-kb *flhA* probe. Primers 8690F (5'-GCTTCG GCTATCTCTGGTTCC-3') and 8690R (5'-GTGTCGTAAGTCGCCTTGATG A-3') were used to generate the ca. 2-kb *motB-flgE* probe. Primers flagF (5'-C ATCAACACCAACACCAGCG-3') and flagR (5'-CATTACTTGAAGAGC GACAGGAT-3') were used to generate the ca. 1.2-kb flagellin probe. A representation of the relationships of the DNA fragments PCR amplified to these genes is given in Fig. 3. These genes were identified and primer sequences were chosen based on sequences available from the *R. capsulatus* genome-sequencing project (<http://wit.IntegratedGenomics.com/IGwit/>).

RESULTS

R. capsulatus ctrA and *cckA* mutants are defective in motility.

When the *ctrA* gene was disrupted (in strain BCKF), motility appeared to be lost (Fig. 2). This defect was complemented when the *ctrA* gene was supplied in *trans* on a plasmid [Fig. 2, BCKF(pCTRA)]. In the *cckA* mutant, BKKR, motility was reduced relative to the wild-type strain, B10, but was not as greatly impaired as in the *ctrA* mutant, BCKF (Fig. 2). Cells spotted onto soft agar plates and incubated aerobically in the dark yielded similar results (not shown).

Comparison of flagellar gene transcript levels in the wild type and in *ctrA* and *cckA* mutants. Because the *R. capsulatus ctrA* gene was shown to be required for transcription of the GTA structural genes (13), we investigated the possibility that the motility phenotypes of the *ctrA* mutant BCKF and the *cckA* mutant BKKR were due to a lack of flagellar gene transcription. Because GTA genes are transcribed maximally in the stationary phase of growth, we evaluated the levels of flagellar gene transcripts at three points over the culture growth cycle (see Materials and Methods). RNA was isolated from wild-type (B10), *cckA* mutant (BKKR), *ctrA* mutant (BCKF), and complemented *ctrA* mutant [BCKF(pCTRA)] strains. These RNA samples were used in RNA blot analyses by hybridization with probes from three different classes of flagellar genes.

We assigned *R. capsulatus* flagellar gene homologues to

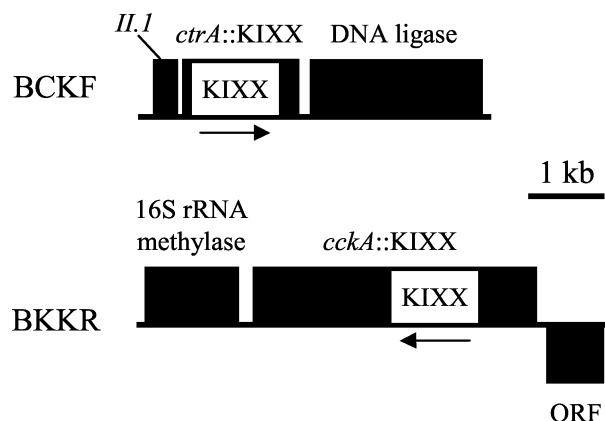


FIG. 1. Map of the chromosomal gene disruptions made in strains BCKF and BKKR. The arrows below the KIXX cartridge indicate the direction of transcription of the *neo* gene. Black boxes drawn above the lines represent genes that are transcribed left to right, and black boxes drawn below the lines represent genes that are transcribed right to left.

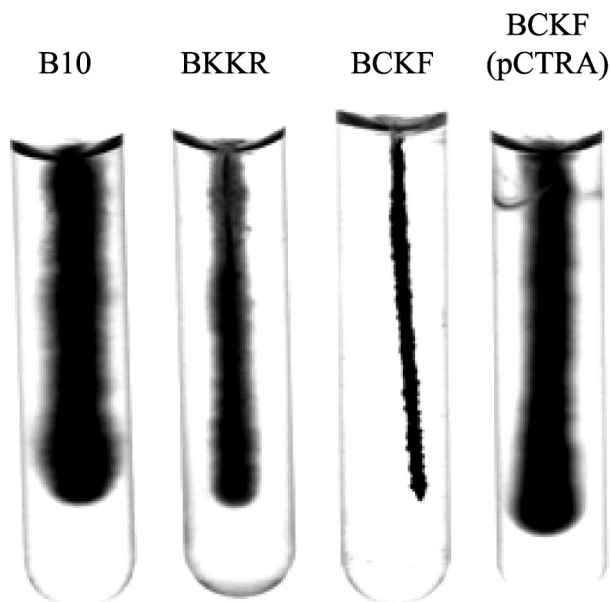


FIG. 2. Motility stabs of *R. capsulatus cckA* and *ctrA* mutants. The strains indicated above each tube were stabbed into YPS soft agar (0.4%) deeps and incubated under photosynthetic conditions for 3 days.

classes of genes (II, III, and IV) that have been established for *C. crescentus* (20), on the assumption that *R. capsulatus* flagellar construction and order of gene expression are as in other bacteria (11, 15, 20).

(i) **Class II gene transcription.** The *R. capsulatus flhA* homologue was used as a representative class II gene probe. The FlhA protein is believed to be part of the export apparatus of the flagellum structure (4). The *cckA* and *ctrA* mutant strains (BKKR and BCKF, respectively) did not contain detectable amounts of these transcripts (Fig. 3A), but the signal was restored when BCKF was complemented with a plasmid-borne copy of *ctrA* [BCKF(pCTRA)].

Interestingly, strain B10 showed a growth phase-dependent transcription pattern for the *flhA* gene (Fig. 3A). The amounts of *flhA* transcripts were highest in the mid-log phase (B10-a) of growth and progressively lower as cultures progressed through the late-log (B10-b) and early stationary (B10-c) phases of growth.

(ii) **Class III gene transcription.** A DNA fragment containing the *R. capsulatus flgE* and *motB* homologues was used as a class III gene probe. The FlgE protein is the major hook protein, and the MotB protein is thought to participate in proton transport to the flagellar motor (4, 15). As was seen with the class II *flhA* transcripts, the *cckA* and *ctrA* mutant strains (BKKR and BCKF, respectively) did not contain detectable levels of the *motB* and *flgE* transcripts, and the signal returned when BCKF was complemented with a plasmid-borne copy of *ctrA* [BCKF(pCTRA)].

Strain B10 showed growth phase-dependent expression of these genes (Fig. 3B), a finding analogous to the result obtained with the class II *flhA* gene probe. However, for these class III genes, the transcript levels appeared to be low in the mid-log phase (B10-a) and higher in the late-log (B10-b) and early stationary (B10-c) phases of growth, in contrast to the results with the class II gene probe (see above).

(iii) **Class IV gene transcription.** For the class IV gene probe, the *R. capsulatus* flagellin-encoding gene was used. Flagellin is the major component of the flagellar filament (4). Similar to the results with the class II and class III genes, the *cckA* and *ctrA* mutant strains (BKKR and BCKF, respectively) did not contain detectable levels of this transcript, and the signal returned when BCKF was complemented with a plasmid-borne copy of *ctrA* [BCKF(pCTRA)].

However, in contrast to the class II and III genes, strain B10 did not show growth phase-dependent transcription of the flagellin gene (Fig. 3C). Shorter exposure times and independent experiments confirmed this result.

When the blots probed with the flagellar genes were overexposed, there was a weak signal visible in the *cckA* mutant and an even weaker signal present in the *ctrA* mutant (data not shown).

DISCUSSION

Figure 2 shows that disruption of the *R. capsulatus ctrA* or *cckA* genes impairs motility in this bacterium: greatly in the *ctrA* mutant and partially in the *cckA* mutant. The data in Fig. 3 indicate that these defects in motility are caused by a reduction in flagellar gene transcription, which correlates with the pattern observed for GTA production: undetectable in *ctrA* mutants and reduced by ca. 90% in *cckA* mutants. We propose that in *R. capsulatus* CckA is involved in GTA and flagellar gene expression through the activation of CtrA (directly or indirectly) by phosphorylation. Because the *cckA* mutant retained low levels of GTA production and motility but the *ctrA* mutant did not, we suggest that, although CckA is the major pathway for activation of CtrA, there is another, minor pathway through which CtrA may be activated less efficiently.

The relative amounts of the three classes of flagellar messages seen on the blots shown in Fig. 3 varied significantly as

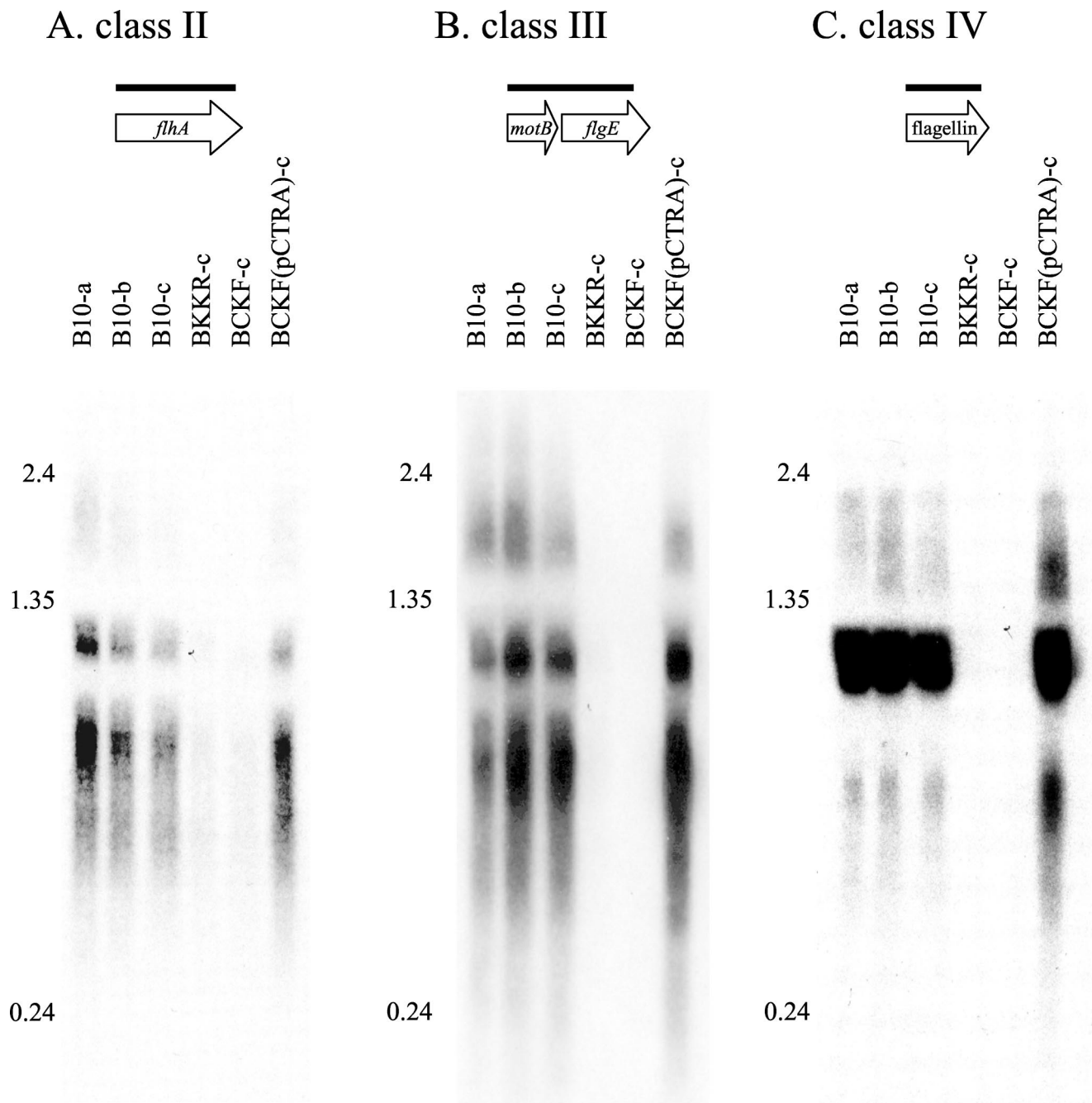


FIG. 3. RNA blot analysis of flagellar gene expression. RNA was isolated from the strains indicated above the lanes at the mid-log (a), late-log (b) or early stationary (c) phases of growth (see Materials and Methods). An equal amount of RNA (8 μ g) was loaded into each lane, and the locations of RNA size standards are shown on the left (in kilobases). The PCR products used as probes are indicated as heavy bars above representations of the genes (arrows). (A) Results when the blot was probed with the *flhA* (class II) probe. (B) Results when the blot was probed with the *motB-flgE* (class III) probe. (C) Results when the blot was probed with the flagellin (class IV) probe.

judged by the length of exposure times required for generation of the images. Compared to the hybridization with the flagellin gene, which required the shortest exposure, the *flgE*-probed blot was exposed 40 times as long and the *flhA*-probed blot was exposed 100 times as long. Although we did not determine the specific incorporation of 32 P into the various probes used, and so these values are not strictly quantitative, the intensities of the signals seen in the RNA blots are consistent with the

predicted relative amounts of the encoded proteins required to assemble the flagellum. The flagellin protein, usually present in $>10^4$ copies per flagellum (4), is the most abundant component. The hook protein FlgE is present at much lower levels (ca. 120 per flagellum) (4). The FlhA protein is thought to be involved in the export apparatus and would therefore be expected to be present in the lowest amount of these three proteins.

It was previously shown that CtrA is required for transcription of the *R. capsulatus* GTA structural genes and that *ctrA* and GTA structural gene transcription is growth phase dependent (13). Comparison of the patterns of GTA and flagellar gene transcription suggests that the regulation of expression of these various sets of genes by CtrA is complicated. Transcription of *ctrA* and the GTA structural genes is maximal in the stationary phase of growth (13), whereas Fig. 3A indicates that *flhA* (class II) transcription peaks in mid-log phase. Therefore, although both the *flhA* and GTA structural genes require CtrA, they have different patterns of transcription over a laboratory culture growth cycle. Furthermore, the levels of class III and IV flagellar gene transcripts exhibited different idiosyncrasies.

It was hypothesized that a stationary-phase signal is transduced through CckA and CtrA to activate GTA gene transcription (13), whereas the results presented here show that the CckA- and CtrA-dependent flagellar genes are regulated differently. The class II *flhA* gene is positively regulated by *ctrA*, because transcript levels were extremely low when *ctrA* was mutated. However, unlike the GTA structural genes, *flhA* appears to be maximally transcribed in the mid-log phase of growth and transcribed less in the stationary phase of growth. One possible explanation for these different patterns is that class II promoters respond positively to a low concentration of CtrA and are inhibited by higher concentrations of CtrA, whereas the GTA genes require a high concentration of CtrA to be activated. This differential responsiveness of promoters is analogous to the way that CtrA functions in *C. crescentus* in the autoregulation of *ctrA* transcription (7). However, it remains to be determined whether CtrA acts directly at the promoters of the *R. capsulatus* genes that we have studied. That is, it is possible that CtrA directly regulates other genes and that the products of these genes then directly regulate the transcription of GTA and flagellar genes.

We tested for growth phase-dependent flagellar gene transcription because of the CtrA-dependent growth phase-dependent GTA gene transcription previously observed (13). We were surprised to find the resultant pattern of transcription, and we do not yet have a full understanding of these results. Nevertheless, one interpretation of the growth phase variation in transcription of the flagellar genes is as follows.

In the mid-log phase of growth, when cells are dividing at the highest rate, there is maximal transcription of the class II genes. When growth slows in the late-log phase of growth, most cells have become replete in class II proteins and so the transcription of class II genes is lower than in mid-log phase, whereas transcription of the class III genes increases. The absence of growth phase-dependent variation in flagellin (class IV) mRNA is surprising given the results seen for the class II and class III genes. We speculate that because the flagellin protein is required in such high amounts, the flagellin gene is continuously transcribed at a high level (if the class II and III genes are expressed) to provide a pool of flagellin for immediate incorporation into the flagellum.

There are precedents for differences in motility over the growth phase of bacterial cultures. In *Bacillus subtilis*, expression of some flagellar genes is controlled by the alternative sigma factor σ^D (9) and is growth phase dependent (8). *E. coli* cells are motile throughout all growth phases, but there are

increases in motility, the expression of flagellar genes, and the production of flagellin that last from the late exponential phase to the early stationary phase of growth (1). While our paper was in review, another study was published that described increases in *E. coli* flagellar gene expression over the growth of a culture (11).

In *C. crescentus*, *ctrA* is thought to be a class I flagellar gene that is required for expression of class II flagellar genes through direct interactions at promoter sequences (6, 19). The results presented here show that the *R. capsulatus ctrA* also appears to be a class I flagellar gene. *C. crescentus* CtrA is an essential protein and performs a variety of functions (18), but several of these functions are not conserved in *R. capsulatus* because the CtrA protein is not essential in this bacterium (13). As a two-component response regulator, CtrA is subject to regulation by phosphorylation that in *C. crescentus* appears to be mediated by a complex phosphorelay network not yet fully understood (18). One part of this network is the essential histidine kinase CckA (10) but, as with *ctrA*, our results indicate that in *R. capsulatus cckA* mutation is not lethal. Nevertheless, a role for these genes in motility as class I flagellar genes is conserved between these two species.

Concluding remarks. Our results indicate that the CckA/CtrA two-component signaling system regulates motility and genetic exchange in *R. capsulatus*, with an increase in both responses as laboratory cultures enter the stationary phase. It is interesting that the production of two such apparently disparate structures, the flagellum and GTA, is controlled by the same regulatory system. However, these two responses actually share physiological and conceptual properties. One physiological similarity is that the flagellum and GTA both are complex protein structures which require assembly and that function outside the cell. Conceptually, both motility and genetic exchange could be viewed as complementary solutions to the problem of a hostile natural environment. An increase in genetic variability provided by GTA-mediated genetic exchange would potentially enable individual members of a population to adapt to an unfavorable environment, whereas an increase in motility would facilitate the swimming of cells to a new, more favorable environment.

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REFERENCES

1. Amsler, C. D., M. Cho, and P. Matsumura. 1993. Multiple factors underlying the maximum motility of *Escherichia coli* as cultures enter post-exponential growth. *J. Bacteriol.* **175**:6238–6244.
2. Barany, F. 1985. Single-stranded hexameric linkers: a system for in-phase insertion mutagenesis and protein engineering. *Gene* **37**:111–123.
3. Beatty, J. T., and H. Gest. 1981. Generation of succinyl-coenzyme A in photosynthetic bacteria. *Arch. Microbiol.* **129**:335–340.
4. Berry, R. M., and J. P. Armitage. 1999. The bacterial flagella motor. *Adv. Microb. Physiol.* **41**:291–337.
5. Brent, R., and M. Ptashne. 1980. The *lexA* gene product represses its own promoter. *Proc. Natl. Acad. Sci. USA* **77**:1932–1936.
6. Domian, I. J., K. C. Quon, and L. Shapiro. 1997. Cell type-specific phosphorylation and proteolysis of a transcriptional regulator controls the G₁-to-S transition in a bacterial cell cycle. *Cell* **90**:415–424.
7. Domian, I. J., A. Reisenauer, and L. Shapiro. 1999. Feedback control of a master bacterial cell-cycle regulator. *Proc. Natl. Acad. Sci. USA* **96**:6648–6653.
8. Haldenwang, W. G. 1995. The sigma factors of *Bacillus subtilis*. *Microbiol. Rev.* **59**:1–30.

9. **Helmann, J. D.** 1991. Alternative sigma factors and the regulation of flagellar gene expression. *Mol. Microbiol.* **5**:2875–2882.
10. **Jacobs, C., I. J. Domian, J. R. Maddock, and L. Shapiro.** 1999. Cell cycle-dependent polar localization of an essential bacterial histidine kinase that controls DNA replication and cell division. *Cell* **97**:111–120.
11. **Kalir, S., J. McClure, K. Pabbaraju, C. Southward, M. Ronen, S. Leibler, M. G. Surette, and U. Alon.** 2001. Ordering genes in a flagella pathway by analysis of expression kinetics from living bacteria. *Science* **292**:2080–2083.
12. **Lang, A. S., and J. T. Beatty.** 2001. The gene transfer agent of *Rhodobacter capsulatus* and “constitutive transduction” in prokaryotes. *Arch. Microbiol.* **175**:241–249.
13. **Lang, A. S., and J. T. Beatty.** 2000. Genetic analysis of a bacterial genetic exchange element: the gene transfer agent of *Rhodobacter capsulatus*. *Proc. Natl. Acad. Sci. USA* **97**:859–864.
14. **LeBlanc, H., A. S. Lang, and J. T. Beatty.** 1999. Transcript cleavage, attenuation, and an internal promoter in the *Rhodobacter capsulatus puc* operon. *J. Bacteriol.* **181**:4955–4960.
15. **Macnab, R. M.** 1996. Flagella and motility, p. 123–145. *In* F. C. Neidhardt, R. Curtiss III, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter, and H. E. Umberger (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology, 2nd ed., vol. 1. ASM Press, Washington, D.C.
16. **Marrs, B. L.** 1974. Genetic recombination in *Rhodospseudomonas capsulata*. *Proc. Natl. Acad. Sci. USA* **71**:971–973.
17. **Ochman, H., J. G. Lawrence, and E. A. Groisman.** 2000. Lateral gene transfer and the nature of bacterial innovation. *Nature* **405**:299–304.
18. **Østerås, M., and U. Jenal.** 2000. Regulatory circuits in *Caulobacter*. *Curr. Opin. Microbiol.* **3**:171–176.
19. **Quon, K. C., G. T. Marczynski, and L. Shapiro.** 1996. Cell cycle control by an essential bacterial two-component signal transduction protein. *Cell* **84**:83–93.
20. **Roberts, R. C., C. D. Mohr, and L. Shapiro.** 1996. Developmental programs in bacteria. *Curr. Top. Dev. Biol.* **34**:207–257.
21. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. *Molecular cloning: a laboratory manual*, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
22. **Scolnik, P. A., and R. Haselkorn.** 1984. Activation of extra copies of genes coding for nitrogenase in *Rhodospseudomonas capsulata*. *Nature* **307**:289–292.
23. **Simon, R., U. Priefer, and A. Pühler.** 1983. A broad host range mobilization system for *in vivo* genetic engineering: transposon mutagenesis in gram-negative bacteria. *Bio/Technology* **1**:37–45.
24. **Solioz, M., and B. Marrs.** 1977. The gene transfer agent of *Rhodospseudomonas capsulata*: purification and characterization of its nucleic acid. *Arch. Biochem. Biophys.* **181**:300–307.
25. **Solioz, M., H.-C. Yen, and B. Marrs.** 1975. Release and uptake of gene transfer agent by *Rhodospseudomonas capsulata*. *J. Bacteriol.* **123**:651–657.
26. **Taylor, D. P., S. N. Cohen, W. G. Clark, and B. L. Marrs.** 1983. Alignment of the genetic and restriction maps of the photosynthetic region of the *Rhodospseudomonas capsulata* chromosome by a conjugation-mediated marker rescue technique. *J. Bacteriol.* **154**:580–590.
27. **Vieira, J., and J. Messing.** 1982. The pUC plasmids, and M13mp7-derived system for insertion mutagenesis and sequencing with synthetic universal primers. *Gene* **19**:259–268.
28. **Wall, J. D., P. F. Weaver, and H. Gest.** 1975. Gene transfer agents, bacteriophages, and bacteriocins of *Rhodospseudomonas capsulata*. *Arch. Microbiol.* **105**:217–224.
29. **Yen, H.-C., N. T. Hu, and B. L. Marrs.** 1979. Characterization of the gene transfer agent made by an overproducer mutant of *Rhodospseudomonas capsulata*. *J. Mol. Biol.* **131**:157–168.