SUPPLEMENTARY INFORMATION

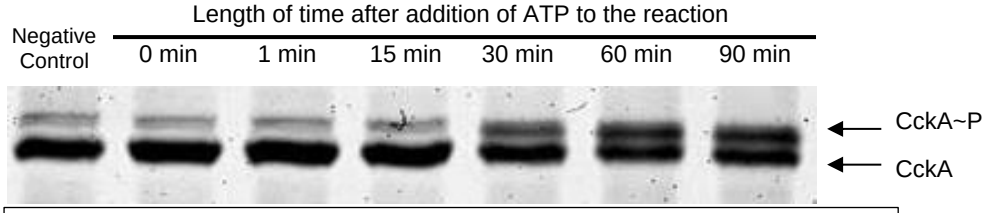

Figure S1. Change in PhosTag gel migration is consistent with ATP-dependent autophosphorylation of CckAΔ69.

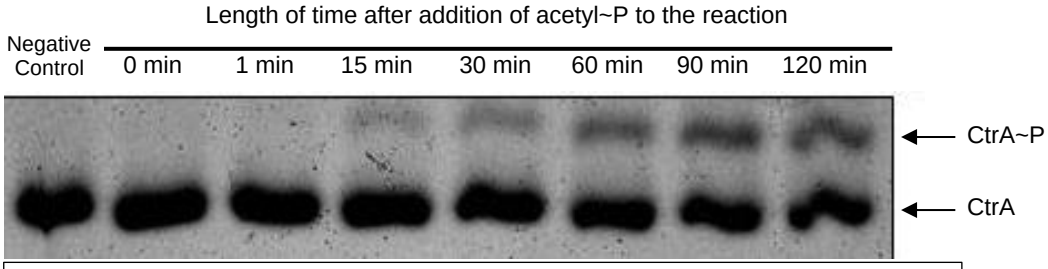

Figure S2. Change in PhosTag gel migration is consistent with acetyl~P-dependent autophosphorylation of CtrA.

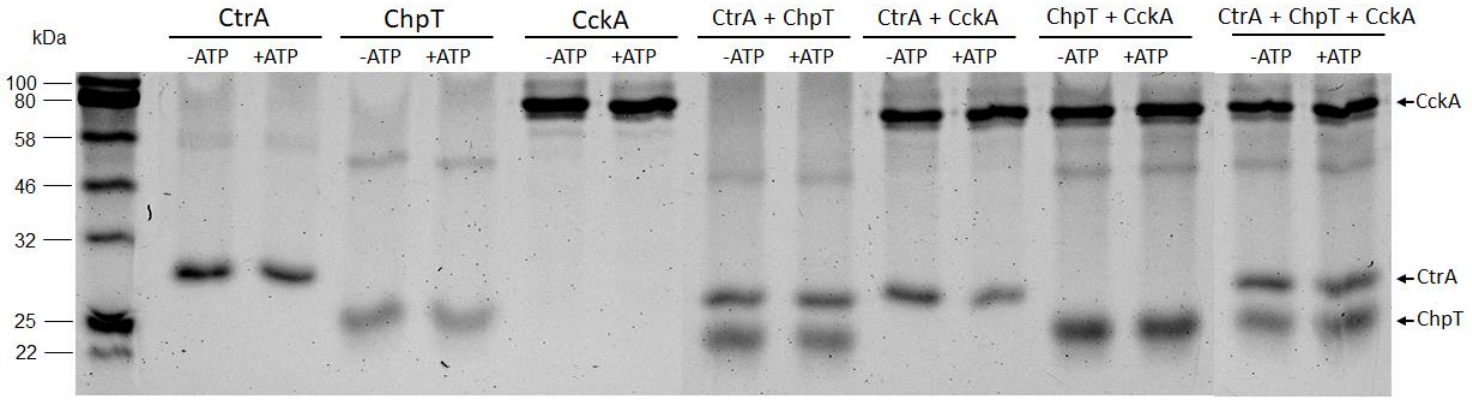

Figure S3. Standard SDS-PAGE of same set of samples used for the PhosTag gel shown in Figure 2. The absence of a change in band migration in samples containing CckA and ATP confirms the interpretation that the change in PhosTag gel mobility results specifically and only from protein phosphorylation.

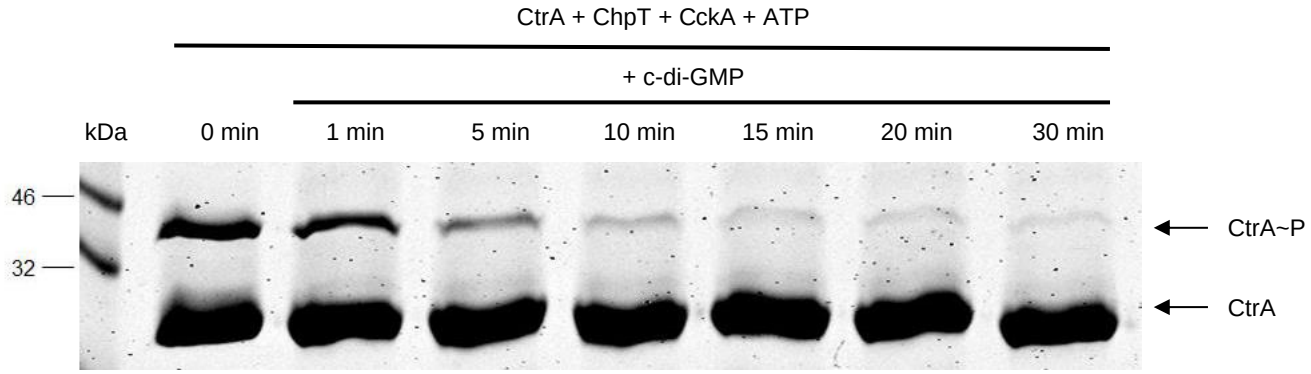

Figure S4. Effect of the addition of c-di-GMP on CtrA phosphorylation in a phosphorelay assay. The presence of c-di-GMP causes a change from phosphorylating CtrA to dephosphorylation of CtrA~P. Samples were removed at the indicated time intervals given in min.

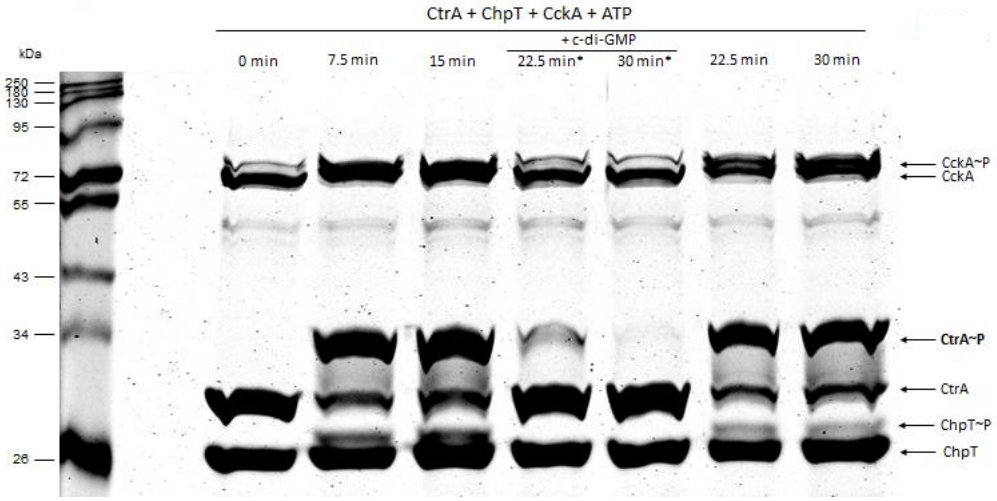

Figure S5. Effect of the addition of c-di-GMP on CtrA phosphorylation in a phosphorelay assay. The presence of c-di-GMP causes a change from phosphorylating CtrA to dephosphorylation of CtrA~P. The rate of dephosphorylation exceeds the rate of phosphorylation under these conditions, and CckA~P is converted to CckA. Samples were removed at the indicated time intervals given in min.

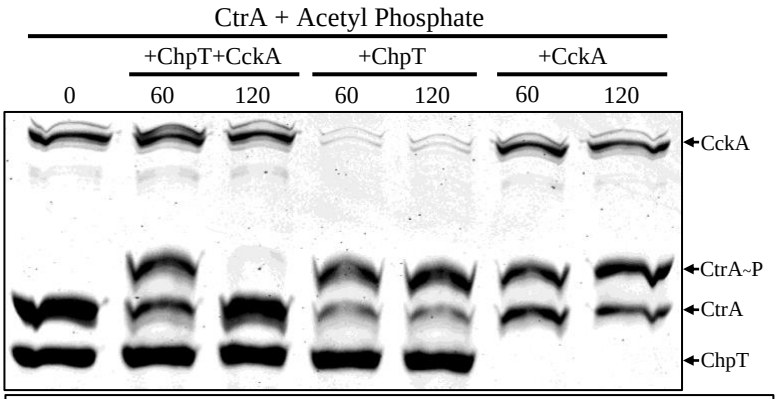

Figure S6. Enzymatic dephosphorylation of CtrA~P requires the complete CckA-ChpT-CtrA phosphorelay because addition of c-di-GMP does not result in dephosphorylation of CtrA~P in the absence of either ChpT or CckA. CtrA was phosphorylated with acetyl~P. Samples were removed at the times indicated in min, with the addition of ADP plus c-di-GMP after 60 min.

```
         270       280         290       300       310       320
C. cre VRLARDPSRIAHLYLYRAEGR--LVAYMIDVSEQKQIELQLSQAQKMQAIGQLAGGVAHD
       : :    .:. .      :::  :.: . :... : .: :. :.:::::::::::::::
R. cap VFLQVTLARVIE------EGRPGLIAVLSDATQLKTLEGQFVQSQKMQAIGQLAGGVAHD
        350              360       370       380       390       400

            330       340       350       360       370       380
C. cre FNNLLTAIQLRLDELLHRHPVGDPSYEGLNEIRQTGVRAADLVRKLLAFSRKQTVQREVL
       ::::::::. . : :. ::  :::.:  :..: :.. :::.:: .:::::::::.. ...
R. cap FNNLLTAISGHCDLLMLRHDKGDPDYTDLDQISQNANRAASLVGQLLAFSRKQTLKPRII
               410       420       430       440       450       460

            390       400       410       420       430       440
C. cre DLGELISEFEVLLRRLLREDVKLITDYGRDLPQVRADKSQLETAVMNLAVNARDAVRAAK
       :: . .:..  :: ::  : : :   .  .:  .:::: ::: ..:::.:::::::.
R. cap DLRDTLSDLTHLLNRLTGEKVVLTLTHDPNLAPIRADKRQLEQVIMNLVVNARDAM---P
               470       480       490       500       510

            450       460         470       480       490       500
C. cre GGGVVRIRTARLTRDEAIQLGFPAAD-GDTAFIEVSDDGPGIPPDVMGKIFDPFFTTKPV
       ::: .::.:  :   : ..   :.  :. . ..:.:.: ::: : .:::::.::.:::
R. cap GGGEIRIETENLHLIEDLKRDRAAVPKGNYVVVKVTDEGVGIPADKLGKIFEPFYTTKKP
       520       530       540       550       560       570

             510       520       530       540       550       560
C. cre GEGTGLGLATVYGIVKQSDGWIHVHSRPNEGAAFRIFLPVYEAPAGAVAVQAVAEPAKPR
       ::::::::.:.::::::. :.:   :  . :. : .::::.. :.     .   ::: :
R. cap GEGTGLGLSTAYGIVKQTGGYIFCDSVLGSGTCFTLFLPAHDRPS-----EIEQEPALPT
       580       590       600       610       620            630
```

Figure S7. Alignment of *C. crescentus* and *R. capsulatus* CckA sequences in the region around the *R. capsulatus* residues H399 and Y589.

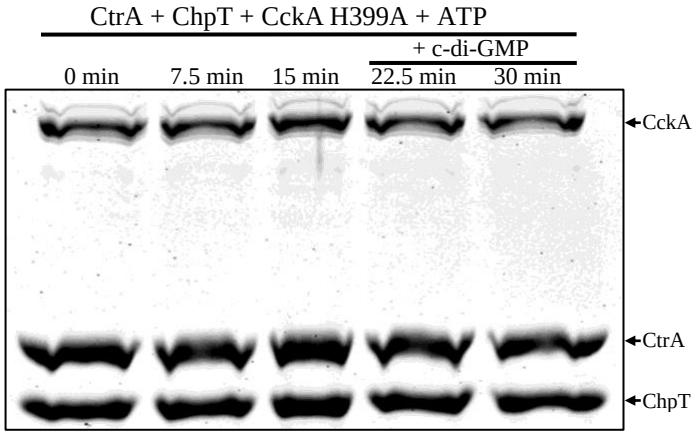

Figure S8. The H399A mutant CckA protein does not autophosphorylate in the presence of ATP. Samples were removed at the indicated time intervals given in min.

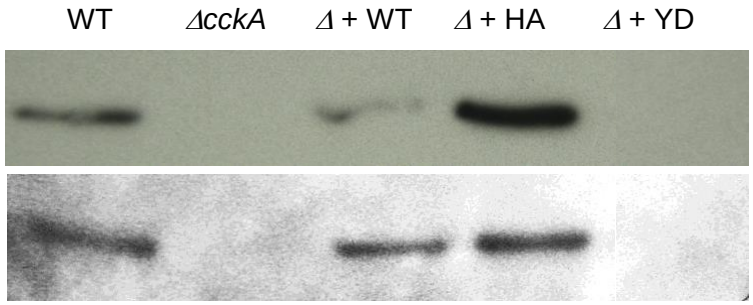

Figure S9. Two Western blots of equivalently-loaded cell-free culture supernatants showing the effects of CckA mutations on release of RcGTA from cells by lysis. Blots were probed with anti-RcGTA capsid protein serum. WT indicates strain SB1003, Δ indicates a deletion of the *cckA* gene, + WT indicates plasmid pRCckA expressing the WT *cckA* gene, + HA indicates plasmid pCW104 expressing the H399A allele of *cckA*, and + YD indicates plasmid pRCckA-YD expressing the Y589D allele. The original images were cropped to remove a lane between the Δ + HA and Δ + YD lanes.

Alignment of ADP-binding region of Cc CckA (from PDB 5IDJ) with Rc CckA

Alignment statistics for Q = Cc CckA and S = Rc CckA

Expect	Identities	Positives	Gaps
3e-32	53/101(52%)	68/101(67%)	4/101(3%)

Cc N131 in PDB 5IDJ = N434 in full length … Cc T195 in PDB 5IDJ = T498 in full-length

```
Cc CckA   1   MNLAVN[131]ARDAVRAAKGGGVVRIRTARLTRDEAIQLGFPAA-DGDTAFIEVSDDGPGIPPD  59
              MNL VN  ARDA+    GGG +RI T  L   E ++    A   G+   ++V+D+G GIP D
Rc CckA   1   MNLVVN[511]ARDAM---PGGGEIRIETENLHLIEDLKRDRAAVPKGNYVVVKVT DEGVGIPAD  57

Cc CckA  60   VMGKIFDPFFT[195]TKPVGEGTGLGLATVYGIVKQSDGWIHVHS  100
               +GKIF+PF+T  TK  GEGTGLGL+T YGIVKQ+ G+I   S
Rc CckA  58   KLGKIFEPFYT[573]TKKPGEGTGLGLSTAYGIVKQTGGYIFCDS  98
```

X = H-bond to ADP via sidechain, X = H-bond to ADP via backbone

Cc N^{131} binds Mg^{2+} in addition to ADP

Figure S10. Alignment of the ADP-binding region of the *C. crescentus* CckA protein with the homologous region of the *R. capsulatus* CckA. The identities in this alignment are 52% and the E-value is $3e^{-32}$. All of the residues found to bind to ADP in the *C. crescentus* crystal structure (PDB accession number 5IDJ) are present in the *R. capsulatus* sequence. Superscript numbers refer to residue number in the *C. crescentus* segment present in PDB 5IDJ, and residue number in the full-length *R. capsulatus* protein.

TABLE S1. Plasmids.

Plasmid	Description	Reference
pET28-a	Expression vector for overexpression of 6-His-tagged proteins in *E. coli*	Novagen
pCckAΔTM	Derived from pET28-a, expresses the C-terminal 6-His-tagged CckA without transmembrane region	This study
pH399A	Derived from pCckAΔTM with mutation at position 399 aa residue to substitute histidine to alanine	This study
pY589D	Derived from pCckAΔTM with mutation at position 589 aa residue to substitute tyrosine to aspartate	This study
pRK415	Broad host-range plasmid pRK415 encoding tetracycline resistance	(1)
pRCckA	Derived from pRK414, contains the WT *cckA* gene transcribed from the native promoter	(2)
pCW104	Derived from pRCckA, contains the H399A mutant *cckA* gene	(3)
pRCckA-YD	Derived from pRCckA, contains the Y589D mutant *cckA* gene	This study

TABLE S2. Primers for PCR amplification and site-directed mutagenesis.

Primer Name	Sequence (5' → 3')
CckA_ΔTM-F	TATACCATGGCGGGGGCGATTTCCGCC
CckA_ΔTM-R	TATAAGCTTGGCCCGCGCCCGGCGG

CckA_H399A-F	GGCGGGGTTGCGGCTGATTTCAACAACTTG
CckA_H399A-R	CAAGTTGTTGAAATCAGCCGCAACCCCGCC
CckA_Y589D-F	CTGGGGCTCTCGACCGCCGACGGGATCGTCAAG
CckA_Y589D-R	CTTGACGATCCCGTCGGCGGTCGAGAGCCCCAG
CckA_Y589D-F #1	GGGGCTCTCGACCGCCGACGGGATCGTCAAGCAG
CckA_Y589D-R #1	CTGCTTGACGATCCCGTCGGCGGTCGAGAGCCCC

REFERENCES

1. Keen NT, Tamaki S, Kobayashi D, Trollinger D. 1988. Improved broad-host-range plasmids for DNA cloning in gram-negative bacteria. Gene 70:191-7.
2. 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 195:5025-40.
3. Westbye AB, Kater L, Wiesmann C, Ding H, Yip CK, Beatty JT. 2018. The Protease ClpXP and the PAS Domain Protein DivL Regulate CtrA and Gene Transfer Agent Production in *Rhodobacter capsulatus.* Appl Environ Microbiol 84.