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Supplemental Information

A Cell-Type-Specific Protein-Protein Interaction Modulates Transcriptional Activity of a Master Regulator in *Caulobacter crescentus*

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Supplemental Figure Legends

Experimental Procedures

Table S1 - Strains and plasmids.

Table S2 - Primers.

Table S3 - Microarray data.

Figure S1

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C.crescentus       MLQQQRTNSRGEKYVIGPTGAPLTLADLPPAETQRWVIRRKAEVVAAVRGGLLSLDEACDRYTLTVEEFLSWQSSIDEHGLAGLRTTRIQQYRH
B.melitensis       MTDLVRPR---IKYVIGPDGSPLTIADLPPANTRRWVIRRKAEVVAAVRGGLLSLEEACQRYKLTTEEFLSWQASIDEYGLAGLRTTRIQQYRH
M.loti             MTDLVRPR---VKYVIGPDGSPLTIADLPPTNTRRWVIRRKAEVVAAVRGGLLSLEEACQKYTLTVEEFLSWQLSIDQHGLAGLRTTQIQEYRH
F.pelagi           MADQVRSR---IKYVIGPDGSPLTLADLPPCNTRRWVIRRKAEVVAAVRGGLLSLEEACSRYTLTTEEFLSWQYSIDQHGLAGLRTTRIQHYRH
M.magnetotacticum  MTETPRPR---VKYVIGPDGSPLTIADLPPVTTRRWVIRRKAEVVAAVRGGLLSLEEACQRYTLTVDEFLSWQFSIDQHGLAGLRTTRIQQYRQ
B.japonicum        MTEPHRPR---VKYVIGPDGSPLTIADLPAPGTKRWVIRRKAEVVAAVRGGLLSLEEACSRYTLTVDEFLSWQFSIDHHGLAGLRTTRIQQYRQ
R.palustris        MTEPHRPR---VKYVIGPDGSPLTIADLPAPGTKRWVIRRKAEVVAAVRGGLLSLEEACSRYTLTVDEFLSWQASIDRHGLPGLRTTRIQHYRQ
X.autotrophicus    MTDAYRPR---VKYVIGPDGGPLTIADLPPPDTRRWVIRRKAEVVAAVRGGLLSLEEACKRYTLTVEEFLSWQSSINDHGLAGLRTTRIQQYRH
A.tumefaciens      MTEMMRPR---VKYVIGPDGSPLTIADLPPANTRRWVIRRKAEVVAAVRGGLLSLEEACERYTLTVEEFLSWQSSINDHGLAGLRTTRIQQYRH
S.meliloti         MTEMMRPR---VKYVIGPDGSPLTIADLPPANTRRWVIRRKAEVVAAVRGGLLSLEEACERYTLTVEEFLSWQSSINSHGLAGLRTTRIQQYRH
R.etli             MTEMIRPR---VKYVIGPDGSPLTIADLPPPNTRRWVIRRKAEVVAAVRGGLLSLEEACERYTLTVEEFLSWQSSINSHGLAGLRTTRIQQYRH
R.leguminosarum    MTEMIRPR---VKYVIGPDGSPLTIADLPPPNTRRWVIRRKAEVVAAVRGGLLSLEEACERYTLTVEEFLSWQSSIEKHGLAGLRATRIQQYRN
S.aggregata        MTEHIRSR---VKYVIGPDGSPLTIADLPPTSTKRWVIRRKAEVVAAVRGGLLSLEEACQRYTLTVEEFLSWQSLIDEHGLAGLRTTRIQHYRH
B.quintana         MTNSIKTQ---MKYVTGPDGSPLTIADLPPKTTRRWVIRRKAEVVAAVRGGLLSLDEACQRYTLTVEEFLSWQSLIDEHGLAGLRITRIQHYRH
B.henselae         MTNLIKTQ---MKYVIGPDGSPLTIADLPPKTTRRWVIRRKAEVVAAVRGGLLSLDEACQRYSLTVEEFLSWQNSIHEHGLAGLRTTRLQQYRY
B.bacilliformis    MTDLIKSQ---MKYVTGPDGSPLTLTDLPPITTRRWVIRRKAEVVAAVKGGLLSLDEACQRYTLTVEEFLSWQRSIERHGLAGLRATRVQQYRQ
P.lavamentivorans  MSEHQISK---VNYVIGPDGSPLTVADLPPPDTKRWVIRRKAEVVAAVRGGLISLDDACRRYSLTVEEFLGWQRSIDRHGLAGLRTTRIQQYR-
M.maris            MQPRLKKE----NYVIGPDGSPLTLGDLPNPSTQRWVVRRKAEVVAAVRGGLLTLEQACQRYSLSEEEFALWRAAVAAHGEKALKVTMIQKYR-
R.sphaeroides      MYLKRVDG---PRQVTLPDGTVLSRADLPPLDTRRWVASRKAAVVKAVIHGLITEREALDRYNLTDEEYEAWERLYARHGAKGLRTTRLQQYR-
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Figure S2

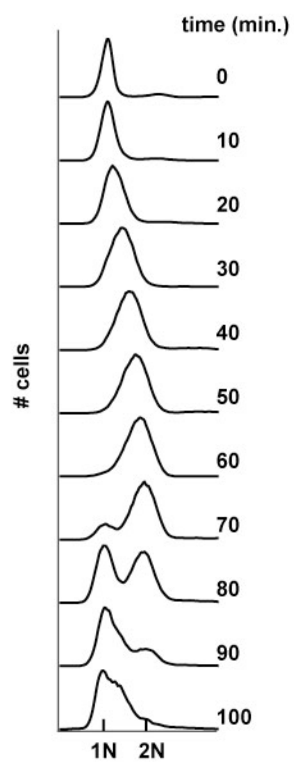


Figure S3

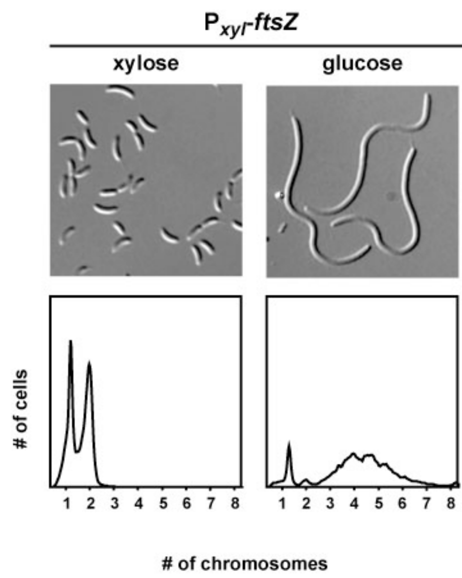
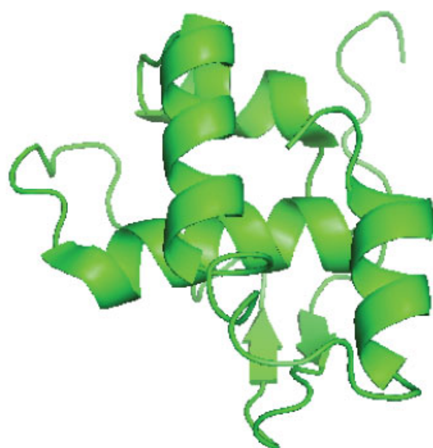


Figure S4

A



B

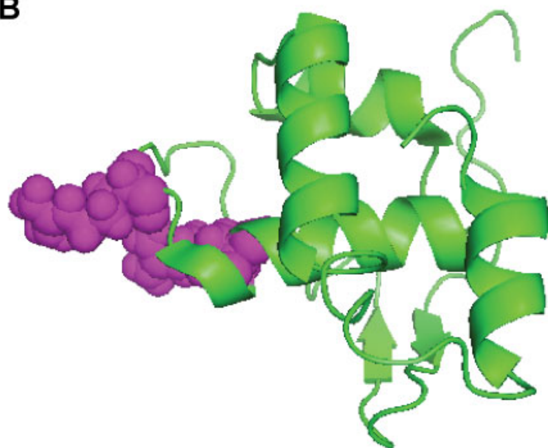
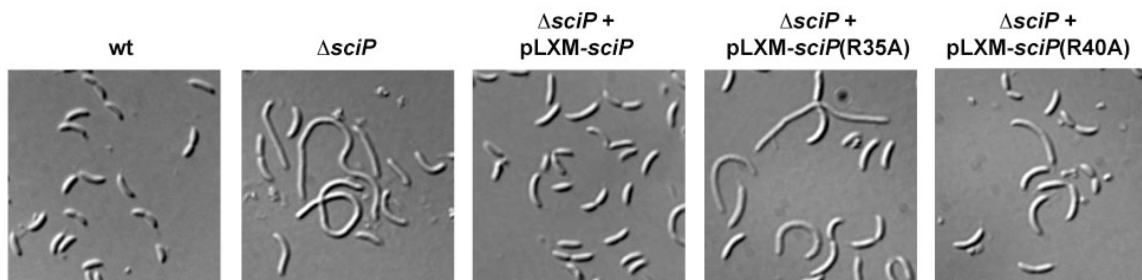


Figure S5

A

strain	cell length (μM)	# cells counted	doubling time (min)
wt	2.48 ± 1.04	332	82
Δ*sciP*	7.16 ± 6.90	312	128
Δ*sciP* + pLXM-*sciP*	2.63 ± 0.88	309	87
Δ*sciP* + pLXM-*sciP*(R35A)	4.92 ± 3.35	315	106
Δ*sciP* + pLXM-*sciP*(R40A)	3.89 ± 1.95	356	94

B



Supplemental Figure Legends

Figure S1. Multiple sequence alignment of SciP orthologs. SciP orthologs from a sampling of alpha-proteobacterial genomes were aligned and conservation visualized using BOXSHADE. Residues shaded in black and grey match or are similar to, respectively, the consensus at that position.

Figure S2. Flow cytometry analysis of cells from Figure 1D. The appearance of new swarmer cells at time 80 min is concurrent with the accumulation of SciP.

Figure S3. Morphology and chromosomal content of *ftsZ* depletion strain. Cells were grown in either xylose to maintain expression of *ftsZ* or in glucose to repress *ftsZ*. Cells were examined by light microscopy (top) or flow cytometry (bottom).

Figure S4. (A) NMR structure (PDB: 2JRT) of a SciP ortholog from *R. sphaeroides.* (B) Residues R35 and R40, which are important for inhibiting CtrA-dependent gene expression are highlighted in magenta with spacefilling.

Figure S5. Complementation analysis for SciP(R35A) and SciP(R40A). (A) Summary of cellular morphology and doubling time in rich media for the strains listed. (B) Micrographs of complementation strains compared to wild type and *ΔsciP*.

Supplemental Experimental Procedures

Bacterial strains and media

E. coli and *C. crescentus* strains were grown as previously described (Skerker et al., 2005). PYE was supplemented with 0.3% xylose or 0.2% glucose when indicated. M2G was supplemented with 0.3% xylose when indicated. Xylose inductions were performed by diluting cultures grown to mid-log phase into PYE supplemented with xylose or adding 0.3% xylose to M2G. For SciP depletion, a culture of ML1750 was grown to mid-log phase, pelleted at 6,800 *g* for 1 minute, washed with PYE, and resuspended in PYE plus glucose. Synchronizations were performed on mid-log phase cells using Percoll (GE Healthcare) density gradient centrifugation.

Cloning and mutagenesis

All expression vectors were constructed using the Gateway cloning system (Invitrogen) as previously described (Skerker et al., 2005). The CC0903 (*sciP*) open reading frame was amplified using the primers listed in Table 2 and cloned into pENTR-D-TOPO to create pENTR-*sciP*. Alanine mutations were made using the QuikChange kit (Stratagene) with pENTR-*sciP* as template. The pENTR-*sciP* plasmids were recombined with pHXM-DEST, pHIS-DEST, pAD-DEST and pBD-DEST to construct the expression plasmids pHXM-*sciP*, pHIS-*sciP*, pAD-*sciP* and pBD-*sciP*, respectively. CC3047 (*rpoD*) was amplified using specific primers to create pENTR-*rpoD* and then recombined with pHIS-DEST to create pHIS-*rpoD*. Entry vectors for *divJ* (cytoplasmic domain), *divK*, *ctrA*, and *phoB* were recombined with pAD-DEST and pBD-DEST.

pAD-DEST was constructed by ligating the Rfa cassette (Invitrogen) into pGAD-c1 linearized with SmaI. pBD-DEST was made by ligating Rfa into pGBDU-c3 that had been digested with ClaI and blunted with the End-It DNA End-Repair kit (Epicentre). The orientation of the Rfa cassettes was verified by EcoRI digestion. pNPTS-spec-DEST was constructed by inserting the Rfa cassette into the SmaI

site of pNPTS138 followed by replacement of the kanamycin resistance cassette (digestion with XbaI and NscI followed by end repair) with the spectinomycin resistance cassette from HP45Ω (excised with SmaI).

The MultiSite Gateway vectors pE1-CC0903_LFR, pE3-CC0903_RFR, and pE2-FRT-tet were constructed by amplifying a 600 bp region upstream of CC0903 (including 15 nucleotides into the coding sequence), a 600 bp region downstream of CC0903 (including the last 30 nucleotides of coding sequence), and the tetracycline resistance cassette from pKOC3 using the primers specified in Table 2 and cloning the PCR products into pDONR221 P1-P4, pDONR221 P3-P2, or pDONR221 P4r-P3r, respectively, by BP recombination (Invitrogen) in a reaction consisting of 40 ng PCR product, 0.5 µL pDONR vector, and 1 µL of BP clonase in a 5.5 µL reaction volume. The BP reactions were incubated overnight at room temperature and then transformed into chemically competent TOP10 cells and plated on LB with kanamycin. The pKO-CC0903 plasmid was then constructed with MultiSite Gateway Pro three fragment recombination between pE1-CC0903_LFR, pE3-CC0903_RFR, pE2-FRT-tet, and pNTPS-DEST in a reaction containing 40 ng of each entry plasmid, 100 ng of the destination vector, and 2 µL of Clonase II enzyme in 10 µL total reaction volume. The recombination reaction was incubated at room temperature overnight and transformed into chemically competent DH5α and plated on LB supplemented with spectinomycin and tetracycline.

The integrating plasmid pX-*sciP* was created by excising GFP from pXGFPN-2 with NdeI and KpnI to replace the GFP cassette with the full *sciP* coding sequence derived by digesting a PCR fragment amplified with primers harboring NdeI and KpnI restriction sites as indicated in Table 2.

The plasmid pEXTAP-*rpoC* for C-terminal TAP-tagging of the chromosomal copy of *rpoC* was generated by amplifying the last 1000 base pairs of the CC0503 open reading frame using the primers specified in Table 2. This PCR product was digested with SalI and NotI and cloned into similarly digested pEXTAP to

create pEXTAP-*rpoC*. This plasmid was then transformed into CB15N with selection on kanamycin.

Deletion and depletion of *sciP*

SciP was deleted through a two-step homologous recombination method previously described (Skerker et al., 2005) using the pKO-CC0903 integrating plasmid. The *sciP* deletion was verified by PCR using one primer outside the region used for homologous recombination and one primer in the tetracycline resistance cassette (Table 2). The tetracycline-marked deletion was transduced into a clean CB15N background using ϕCR30.

To generate a depletion strain, the integrating plasmid pX-*sciP* was transformed into CB15N by electroporation and integrants of *sciP* at the *xylX* locus were selected on PYE plates containing kanamycin and glucose. The tetracycline-marked *sciP* deletion was then transduced from ML1749. The depletion strain was selected on PYE plates containing kanamycin, oxytetracycline, and xylose and verified by PCR to contain *sciP* only at the *xylX* locus.

Protein purification and antibody production

His_6-SciP was expressed from pHIS-*sciP* plasmid and purified, along with His_6-CtrA, as described previously (Skerker et al., 2005). His_6-RpoD was expressed from pHIS-*rpoD* and purified as with His_6-SciP and His_6-CtrA but with the addition of 6 M GuHCl to the lysis buffer. RpoD was bound to Ni-NTA resin under denaturing conditions and subsequently renatured by washing in wash buffer with no GuHCl. Refolding was confirmed by CD spectroscopy. TAP-tagged RNA polymerase was purified from ML1799 as previously described (Rigaut et al., 1999) and dialyzed overnight at 4°C against 500 volumes of TGED buffer (10 mM Tris-HCL pH 7.9, 50% glycerol, 0.1 mM EDTA, 0.1 mM DTT, 500 mM NaCl). Coomassie staining was used to verify that purified TAP-tagged RNA polymerase contained alpha, beta, beta', and sigma subunits. Rabbit polyclonal antisera were generated from purified His_6-SciP (Covance).

Pulse-chase and *in vivo* phosphorylation analyses

Cultures grown in M2G were and for ML1748, pre-induced with xylose for one hour and labeled with [^{35}S]-methionine and chased with 1 mM methionine and 0.3% casmino acids. 1 mL aliquots were removed at indicated time points, cells were pelleted by centrifugation at 20,000 x g, and frozen in liquid nitrogen. Cell pellets where resuspended in 50 µL of lysis buffer (10 mM Tris pH 8, 1% SDS, 1mM EDTA), boiled for 2 minutes to lyse, and diluted with 800 µL chilled IP buffer (50 mM Tris pH 8, 150 mM NaCl, 0.5% Triton X-100). Samples were pre-cleared with 20 µL Pansorbin Cells (Calbiochem) immunoprecipitated with 1.5 µL CtrA antibody at 4 C over night, and immune complexes were collected with 30 µL protein A beads (Invitrogen). The samples were washed 3x with chilled IP buffer and eluted in 30 µL of 2x SDS sample buffer by boiling for 5 min. The samples were loaded onto 10% Tris-HCl gels (Bio-Rad) and run at 150 V for 40 min at room temperature. Gels transferred to Whatman paper, dried for 1 hr and exposed to a phosphor screen for 72 hours.

Analysis of CtrA phosphorylation *in vivo* was performed essentially as described previously (Domian et al., 1997). Briefly, one colony was inoculated into M5G medium containing 0.05 mM phosphate and grown overnight at 30°C. The cultures were grown at 30°C until the optical density of the culture measured at 660 nm was in the range 0.1 to 0.2. Cultures were then split with one maintained in glucose and the other grown in xylose for 2 hours. Equal numbers of cells (based on OD) were labeled for 5 minutes with 30 µCi/mL [γ-^{32}P]ATP before immunoprecipitating CtrA.

Electrophoretic mobility shift assays

For each indicated DNA probe, PAGE purified primers (Sigma-Genosys) were annealed in TEN buffer (10 mM Tris-HCl (pH 8.0), 1 mM EDTA, and 50 mM NaCl) at 95°C for 5 minutes and slowly cooled to room temperature. The double-stranded DNA was labeled with [γ^{32}P]-ATP using T4 polynucleotide kinase (NEB). 10 µM CtrA was phosphorylated using 1µM MBP-EnvZ in HKEG buffer

(10 mM HEPES-KOH [pH 8.0], 50 mM KCl, 10% glycerol, 0.1 mM EDTA, 1 mM DTT) plus 5 mM $MgCl_2$ and 1 mM ATP for 20 min at 37°C. CtrA~P was diluted into HKEG plus $MgCl_2$ to 10X before being added to the gel shift reactions. TAP-tagged RNA polymerase was pre-incubated at 2.8 μM with an equal volume of 14 μM RpoD for 1 hr at 4°C. Proteins were pre-incubated at room temperature for 30 min in gel-shift buffer (50 mM KCl, 5mM $MgCl_2$, 20 mM Tris HCl (pH 8.0), 100 μM EDTA, 1 mM DTT, 1 mg/ml BSA, 10% glycerol, 100 ng/ml competitor DNA (Poly (I)·Poly(C))) and then incubated with 3.5 nM or 0.7 nM labeled DNA at room temperature for 30 min in a 18 μl reaction volume. 2 μl of 0.2% bromophenol buffer was added to each reaction immediately before the samples were loaded onto a Novex 6% DNA retardation gel (Invitrogen) or Ready Gels 5% TBE gels (Biorad) and run at 300 V in chilled 0.5x TBE buffer for approximately 17 min. Gels were transferred to Whatman paper, covered with Saran wrap, dried for 1 hr, exposed to a phosphor screen for 1 hr, and then scanned with a Typhoon scanner.

Quantitative real-time PCR for ChIP and expression analysis

Quantitative real-time PCR for ChIP and expression analyses was performed using the DNA Engine Opticon 2 system (MJ Research) and the QuantiFast SYBR Green PCR Kit (Qiagen). Each reaction contained 2.5% of total ChIP sample or 2% total cDNA for expression analysis, 10 pmol of forward and reverse primer, 1x Quantifast SYBR Green PCR master mix (Qiagen) in a 25 μl reaction. Samples were amplified by incubating at 50°C for 2 min, 95°C for 15 min followed by 45 cycles of 95°C for 15 sec, and 60°C for 30 sec followed by fluorescence data collection. Cycle threshold (Ct) values were calculated using Opticon Monitor software (MJ Research).

Bacterial ChIP was performed as described previously using 2.5 μL of polyclonal α-CtrA antibody for each pull-down (Radhakrishnan et al., 2008). The % input values for the ChIP samples were calculated using the average Ct values from triplicate measurements and a standard curve generated from five two-fold serial

dilutions of the input samples. Each ChIP experiment was performed on three independent colonies to calculate standard error bars. The relative expression of genes was calculated using the following formula: ratio = $((E_{target})^{\Delta CP_{target}(control-sample)}) / ((E_{rho})^{\Delta CP_{rho}(control-sample)})$. For expression analysis, 1 μg of total RNA was reverse transcribed into cDNA using Superscript-II, RNA template degraded was with RNAse H, and nucleotides were removed using the QIAquick PCR purification kit (Qiagen).

Supplemental References

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