Supporting Information

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SI Materials and Methods

Growth Conditions. *M. xanthus* was grown at 33 °C in casitone-Tris (CTT) medium, and *E. coli* at 37 °C in Luria broth. When required, the growth media were supplemented with vitamin B_{12} (Sigma-Aldrich) as described previously (1). Concentrations of cobalamin stocks in aqueous buffer were estimated by absorbance using the following extinction coefficients: ε ($M^{-1}cm^{-1}$) of 27,500 at 361 nm for CNB_{12}, 8,200 at 522 nm for $AdoB_{12}$, and 9,100 at 520 nm for MeB_{12} (2). *T. thermophilus* HB8 was grown as recommended (DSMZ) and genomic DNA was isolated using the Wizard kit (Promega). Overexpression of intein and His_6-tagged proteins was induced overnight at 18 °C and 25 °C, respectively.

Strain Construction and Phenotypic Analysis. CarH and CarA coding segments were amplified by PCR using pMR2830 and pMR2838, respectively (Table S2). *TtcarH* was PCR-amplified from genomic DNA with appropriate primers, cloned into the NdeI and BamHI sites of pET15b, and used to PCR-amplify the TtCarHCt coding sequence. Hybrid genes encoding chimeric proteins were obtained by PCR overlap extension (3), and cloned into the 5′ KpnI and 3′ EcoRI sites of pMR2828 (Table S2), where they are expressed from a constitutive promoter (1, 4). These plasmid constructs have a 1.38-kb *M. xanthus* DNA fragment for chromosomal integration by homologous recombination, and a kanamycin resistance (Km^R) marker for positive selection. Every plasmid construct was verified by DNA sequencing and electroporated into the Δ*carA* Δ*carH M. xanthus* strain (MR1716; Table S2), and selected for chromosomal integration by growth on CTT plates with 40 μg/mL Km. pMR3184 has an insert for generating in-frame *carS* deletions (5), a positive selection Km^R marker, and a galactose sensitivity gene (Gal^S) for negative selection. It was introduced into the Δ*carA,* Δ*carH*, or Δ*carA* Δ*carH M. xanthus* strains obtained previously (MR844, MR848, and MR1716, respectively; Table S2). Strains with the Δ*carS* allele were generated by allelic exchange (1), and verified by PCR analysis. To obtain a complete in-frame deletion of the *M. xanthus pduO* gene, we first constructed plasmid pMR3425, with approximately 0.91 kb and 0.93 kb of genomic DNA immediately upstream and downstream of *pduO*, respectively. pMR3425 (Table S2), which also contains Km^R and Gal^S markers, was introduced into the Δ*carA M. xanthus* strain and haploid colonies with the Δ*pduO* allele (as well as the Δ*carA* one), obtained following procedures previously described (1), were identified by PCR. To assay the color phenotypes, cells were streaked or spotted (8 μL cell droplets of exponentially growing cultures at OD_{550} of 0.8) on CTT plates and grown for 2 d in the dark or in the light with or without 1 μM CNB_{12} in the growth medium. The *carB*::Tn*5-lac*-132 P_B-*lacZ* reporter probe (conferring tetracycline resistance) was introduced into a given strain, and its expression level was estimated in terms of β-gal–specific activity (in nmol of *o*-nitrophenyl β-D-galactoside hydrolyzed/min/mg protein). Reporter *lacZ* expression in the dark and in the light, and in the presence or absence of B_{12} was carried out as described (1). Basically, a starter culture of the *M. xanthus* strain under study was grown in the dark in 10 mL CTT supplemented with the required antibiotic. This culture was added to two separate culture flasks (0.25 mL each) containing 12 mL fresh CTT and antibiotic, with 1 μM CNB_{12} included in one of the two flasks. After 16 h growth in the dark at 33 °C, each 12-mL culture was split into two, one of which remained in the dark and the other exposed to light, while growth continued for another 8 h.

Light Irradiation. Unless otherwise specified, plates or liquid cell cultures exposed to light were illuminated with white light from three 18-W fluorescent lamps at an intensity of approximately 10 W/m^2. For illumination with red light (at approximately 12 W/m^2), cells were exposed to light from four 100-W incandescent lamps passed through a Supergel number 26 red filter (Rosco). Protein samples in 1.5 mL tubes were irradiated for 5 min with light or red light (as described earlier), or with light centered at wavelengths of 360 nm (12 W/m^2), 438 nm (15 W/m^2), and 540 nm (11 W/m^2) using UV-2E/C, blue-light CFP, and G-2E/C filter sets, respectively, in a Nikon Eclipse 80i epifluorescence microscope. Light intensities were estimated by using an 1815-C optical power meter equipped with an 818-SL detector (Newport).

Two-Hybrid Analysis. The *E. coli* two-hybrid system used is based on functional complementation of the T25 and T18 fragments of the *Bordetella pertussis* adenylate cyclase catalytic domain when two test proteins interact (6). Coding regions of interest were PCR-amplified and cloned into the XbaI and BamHI sites of pKT25 or pUT18C. Given pairs of these constructs were introduced into *E. coli* strain BTH101 (*cya*$^-$) by electroporation. Negative controls were pairs with only one fusion protein expressed, and a positive control was the GCN4 leucine zipper. Interaction was assessed qualitatively from the blue color developed on LB plates containing 40 μg/mL X-Gal with or without 1 μM CNB_{12} in the dark or light, and quantitatively by the β-gal–specific activity measured in three independent experiments for each interacting pair tested (6).

Protein Purification. His_6-tagged TtCarH overexpressed using pET15b was purified under native conditions using TALON metal affinity resin (Clontech) and imidazole elution. Other proteins were overexpressed using pTYB12 constructs as fusions to intein, which was subsequently removed by intramolecular cleavage (leaving an N-terminal AGH) and purified with the IMPACT kit and accompanying protocols (New England Biolabs). MonoS ion-exchange chromatography was used in a final purification step. Protein concentrations were determined using the BioRad protein assay kit, or absorbance at 280 nm with $ε_{280}$ ($M^{-1}cm^{-1}$) of 30,940 (CarA, CTt1), 21,220 (CarACt), 20,970 (TtCarHCt and its H177A variant), and 32,430 (CTt2). Absorption spectra were recorded in the absorbance mode of a Pistar spectropolarimeter (Applied Photophysics) or a DU-640 spectrophotometer (Beckman).

DNA-Binding Assays. EMSA and DNase I footprinting assays under similar solution conditions were performed in the dark or after light irradiation (as described earlier) using as 5′-end ^{32}P-labeled PCR-amplified DNA probes: (*i*) a 130-bp probe (CCR130) for CarA, CTt1, and CTt2 (Fig. S2*A*); and (*ii*) a 177-bp probe for TtCarH (Fig. S6*A*). A 20-μL reaction volume containing the DNA probe (1.2 nM, approximately 13,000 cpm), protein, and, as required, the specified cobalamin (final concentrations as indicated), 100 mM KCl, 25 mM Tris, pH 8, 1 mM DTT, 10% glycerol, 200 ng/μL BSA, and 1 μg of sheared salmon sperm DNA as nonspecific competitor was incubated at 37 °C for 30 min. EMSA reactions were performed, and the gels were loaded and run, in a dark room in which very dim stray light aided in viewing. Samples were covered with aluminum foil during the 30-min incubation period. The electrophoresis was carried out in the dark (inside a cooling chamber) for 1.5 h at 200 V, 10 °C in an 6% nondenaturing polyacrylamide gel (37.5:1 acrylamide:bisacrylamide) that was prerun for 30 min in 0.5× TBE buffer

(45 mM Tris base, 45 mM boric acid, 1 mM EDTA). Identical reaction conditions were used in DNase I footprinting assays except that 10 mM $MgCl_2$ was added to the incubation buffer. As with EMSA, DNase I footprinting reactions were performed in a dark room with very low light. After incubating the DNA probe with protein in the absence or presence of a given cobalamin, the mix was treated with 0.07 units of DNase I for 2 min at 37 °C and quenched with EDTA. When the reaction had been stopped, it was not necessary to maintain the dark conditions, and sample loading onto the gel was carried out under normal light. DNA was ethanol-precipitated and run in 8 M urea 6% polyacrylamide gels against G+A and C+T chemical sequencing ladders of the DNA probe used. EMSA and DNase I footprinting assays were carried out for each experimental condition three to 10 times. Gels were vacuum-dried and analyzed by autoradiography.

SEC. An AKTA HPLC unit and a Superdex200 analytical size-exclusion column (GE Life Sciences) equilibrated with 150 mM NaCl, 50 mM phosphate buffer, pH 7.5, and 2 mM β-mercaptoethanol was used for SEC. The column was calibrated by using the following as standards (Sigma-Aldrich): aprotinin (6.5 kDa), cytochrome C (12.4 kDa), carbonic anhydrase (29 kDa), ovalbumin (43 kDa), BSA (66 kDa), yeast alcohol dehydrogenase (150 kDa), β-amylase (200 kDa), blue dextran (2 MDa, to determine the void volume V_o), and vitamin B_{12} (1.35 kDa, to estimate total bed volume, V_t). The calibration curve obtained was: $\log M_r = 7.885 - 0.221V_e$, where M_r is the apparent molecular weight and V_e is the elution volume. Protein (100 μL; 45–165 μM), pure or incubated with a fivefold molar excess of a given cobalamin for at least 15 min in the dark, was injected into the column or, if required, subjected to light irradiation as described earlier, just before injection. Elution at 0.4 mL/min flow rate was tracked by absorbance at 280, 361, and 522 nm. Peak fractions were collected and analyzed for M_r (from V_e), and checked by SDS/PAGE. The reported M_r and the error correspond to the mean of a minimum of three independent measurements and SEM, respectively. The protein concentration in the peak was estimated using the BioRad protein assay and that of $AdoB_{12}$ from the absorbance at 522 nm.

Chemical Cross-Linking. For chemical cross-linking in the dark at room temperature, 25 mM of freshly prepared dithiobis(succinimidylpropionate) (Pierce) in DMSO was added to a final concentration of 1 to 2.5 mM to 5 μM pure protein in 150 mM NaCl, 50 mM phosphate buffer, pH 7.5 (total volume 100 μL). After 1 h incubation, cross-linking was quenched with 1 M Tris, pH 7.5 (150 mM final concentration), for another 15 min. Samples in Novex LDS-PAGE loading buffer were analyzed under nonreducing conditions by SDS/PAGE by using 10% NuPAGE Bis-Tris precast gels with Mes buffer (Invitrogen). The reproducibility of the cross-linking data was checked in three independent experiments.

ITC. ITC was carried out in a VP-ITC system (MicroCal). ITC buffer (100 mM NaCl, 50 mM phosphate buffer, pH 7.5) was used in TtCarHCt dialysis and for preparing cobalamin stock solutions. Concentrations were determined as described earlier. Samples were degassed before ITC. TtCarHCt (1.44 mL; 18 μM; or buffer alone, to correct for the heat of dilution of the titrant) in the ITC cell was titrated with 30 injections of 8 μL of a given cobalamin (65–75 μM) in the dark or after irradiation with light (as described earlier) at 25 °C with stirring (300 rpm). Heat changes were detected only in titrations of TtCarHCt with $AdoB_{12}$, and data from four independent experiments were analyzed. Each of these, after correcting for the heat of dilution of the titrant, was fit to a single-site binding model using MicroCal Origin software to estimate the binding stoichiometry (N), enthalpy (ΔH), entropy (ΔS), and equilibrium association constant (K_a) and the corresponding errors for each parameter.

1. Pérez-Marín MC, Padmanabhan S, Polanco MC, Murillo FJ, Elías-Arnanz M (2008) Vitamin B_{12} partners the CarH repressor to downregulate a photoinducible promoter in *Myxococcus xanthus*. *Mol Microbiol* 67:804–819.
2. Hill JA, Pratt JM, Williams RJP (1964) The chemistry of vitamin B_{12}. Part I. The valency and spectrum of the coenzyme. *J Chem Soc (Lond)* 5149–5153.
3. Horton RM, Hunt HD, Ho SN, Pullen JK, Pease LR (1989) Engineering hybrid genes without the use of restriction enzymes: Gene splicing by overlap extension. *Gene* 77: 61–68.
4. Pérez-Marín MC, López-Rubio JJ, Murillo FJ, Elías-Arnanz M, Padmanabhan S (2004) The N terminus of *Myxococcus xanthus* CarA repressor is an autonomously folding domain that mediates physical and functional interactions with both operator DNA and antirepressor protein. *J Biol Chem* 279:33093–33103.
5. León E, et al. (2010) A bacterial antirepressor with SH3 domain topology mimics operator DNA in sequestering the repressor DNA recognition helix. *Nucleic Acids Res* 38:5226–5241.
6. Karimova G, Ullmann A, Ladant D (2000) A bacterial two-hybrid system that exploits a cAMP signaling cascade in *Escherichia coli*. *Methods Enzymol* 328:59–73.

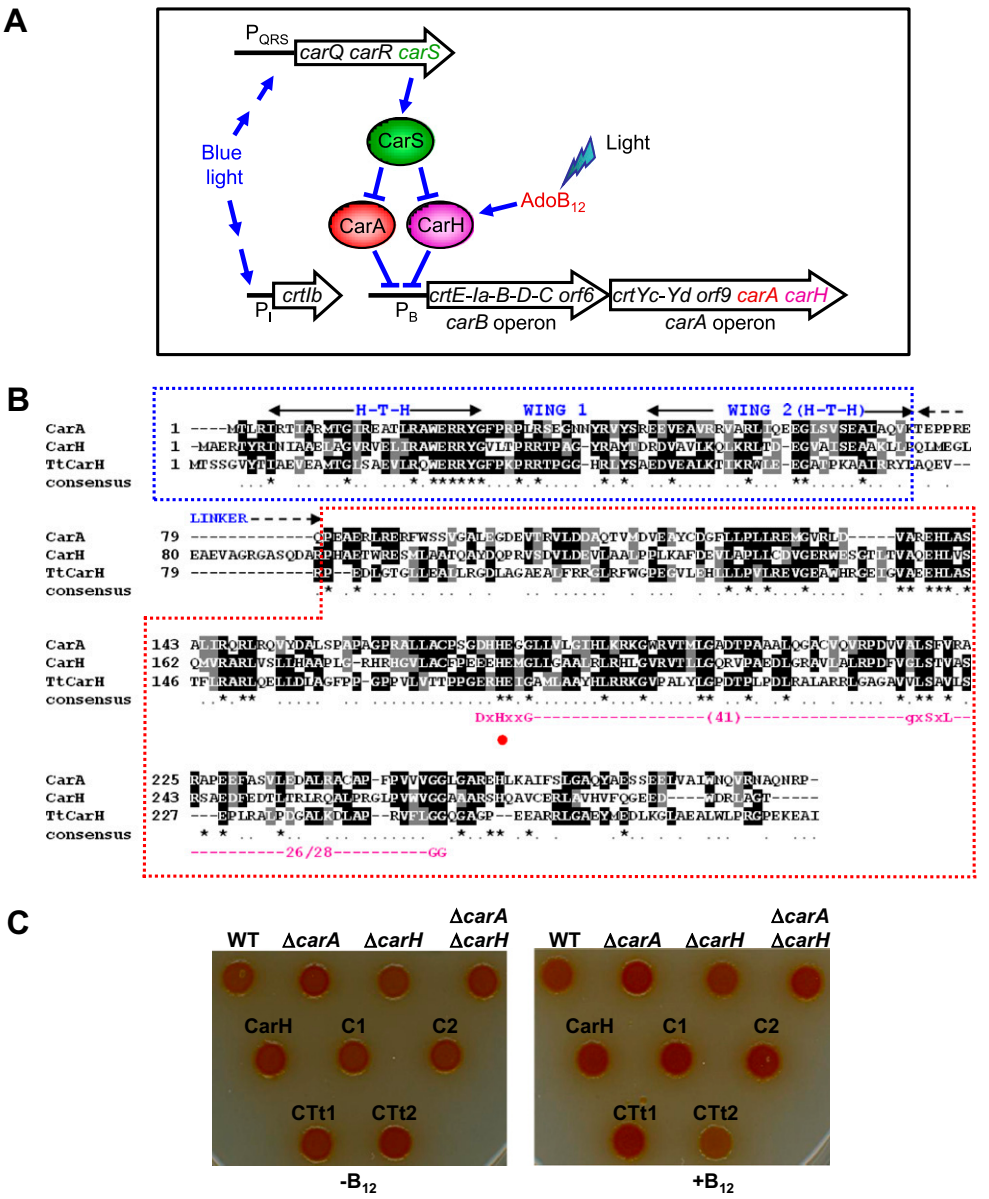

Fig. S1. (*A*) Scheme summarizing the gene regulatory network for light-induced carotenogenesis in *M. xanthus*. For simplicity, the network shows only the factors and interactions mentioned in the text. Light activates transcription at promoters P_{QRS}, P_I, and P_B, which drive expression of the regulatory *carQRS* operon, of the carotenogenic gene *crtIb*, and of the carotenogenic *carB* operon, respectively. Transcription of the *carA* cluster, which includes the carotenogenic *crtYc-Yd* genes, occurs from a light-independent promoter as well as via transcriptional readthrough from P_B (1). P_B is repressed in the dark by CarA, or by CarH in the presence of $AdoB_{12}$. The CarS antirepressor, expressed from P_{QRS} in the light, mimics operator to physically target the DNA-binding domain of CarA or CarH to relieve repression of P_B. Colony color is red when transcription at both P_I and P_B is up-regulated, and orange when only P_B is induced. (*B*) Sequence alignment of CarA, CarH, and TtCarH [National Center for Biotechnology Information (NCBI) accession numbers CAA79964, CAA79965, and YP_006039, respectively] highlighting the two domains and the linker between them. Dotted lines in blue demarcate the N-terminal domain and those in red the C-terminal one. The helix–turn–helix (H-T-H) and wings (wing 1 and wing 2) determined for CarANt (2) are shown, as is the linker region and the consensus B_{12}-binding motif from methionine synthase and related enzymes (3–5). Residues are shaded black if identical and gray if similar in at least two of the aligned sequences. Residues conserved in all three sequences are indicated by asterisks in the consensus line. The red dot points to the conserved H mutated to A that was examined in this study. (*C*) Colony color phenotype for the indicated *M. xanthus* strains in the light without ($-B_{12}$) and with 1 μM CNB_{12} ($+B_{12}$) present.

1. Elías-Arnanz M, Fontes M, Padmanabhan S (2008) Carotenogenesis in *Myxococcus xanthus*: A complex regulatory network. Myxobacteria: Multicellularity and Differentiation, ed Whitworth DE (ASM Press, Washington, DC), pp 211–225.
2. Navarro-Avilés G, et al. (2007) Structural basis for operator and antirepressor recognition by *Myxococcus xanthus* CarA repressor. *Mol Microbiol* 63:980–994.
3. Drennan CL, Huang S, Drummond JT, Matthews RG, Ludwig ML (1994) How a protein binds B_{12}: A 3.0 A X-ray structure of B_{12}-binding domains of methionine synthase. *Science* 266: 1669–1674.
4. Ludwig ML, Drennan CL, Matthews RG (1996) The reactivity of B_{12} cofactors: The proteins make a difference. *Structure* 4:505–512.
5. Ludwig ML, Matthews RG (1997) Structure-based perspectives on B_{12}-dependent enzymes. *Annu Rev Biochem* 66:269–313.

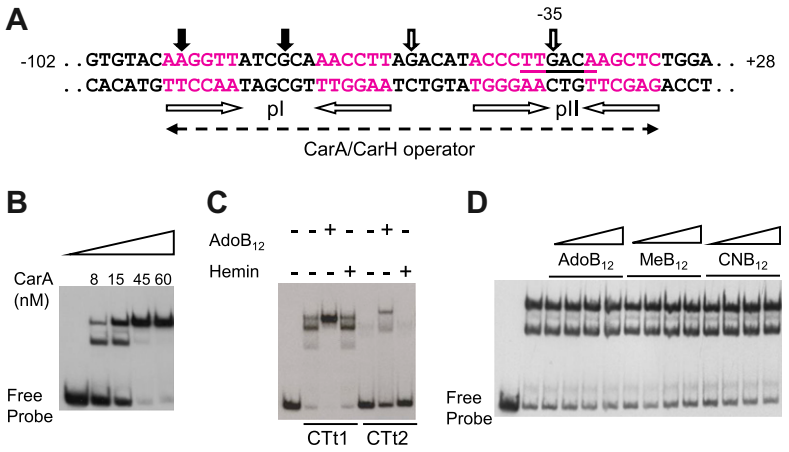

Fig. S2. The CarA/CarH operator probe and EMSA analysis with this probe. (*A*) Schematic of the 130-bp DNA probe, CCR130, spanning the CarA/CarH operator probe and the *carB* promoter region; numbers are relative to the transcription start site (+1). The −35 segment of the P_B promoter is underlined. The high-affinity binding site pI (−64 to −47) and the low-affinity one pII (−40 to −26) are shown by the two unfilled horizontal arrows facing head-on, with the palindromic sequences in magenta. Vertical arrows indicate the DNase I hypersensitive sites established previously (filled) (1); and in Fig. 2*C*. (*B*) EMSA showing the progressive, step-wise, binding to CCR130 for increasing concentrations of CarA. (*C*) EMSA in the dark with CCR130 and 25 nM CTt1 or CTt2 alone, or with 100 nM $AdoB_{12}$ or hemin, as indicated. (*D*) EMSA with CarA (20 nM) and CCR130 in the presence of increasing amounts of different cobalamins, as labeled. Leftmost lane is probe alone and the one immediately to its right has no B_{12} added. Increasing $AdoB_{12}$, MeB_{12}, or CNB_{12}, concentrations (in nM) are 16, 31, 63, and 125.

1. López-Rubio JJ, et al. (2004) Operator design and mechanism for CarA repressor-mediated down-regulation of the photoinducible *carB* operon in *Myxococcus xanthus*. *J Biol Chem* 279: 28945–28953.

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EcPduO       1 ------------------------------------------------------------MAIYTRTGDSCSTSLFTGQRVSKTH
MxPduO       1 ------------------------------------------------------------MKIYTKSGDACETCLFCGCRVAKDD
HsMMAB       1 MAVCGLGSRLGLGSRLGLRGCFGAARLLYPRFQSRGPQGVEDGDRPQPSSKTPRIPKIYTKTGDKCFSSTFTGERRPKDD
consensus    1                                                          ..***..** * ...*.* *. *..

EcPduO      26 LRVETYGTTDELNATLSLCYCATAIE--SHRILLEAIQQQIPWFSAELAS-ESEQPSAQLRY--IGTEEHAALENAIDSA
MxPduO      26 VRVDAYGEVDELNATLGLVRSFECPP--DVDALLHRLQDQLPTVGAVLATPEGTKASAHIPE--LKAEWAEDMERAIDGF
HsMMAB      81 QVFEAVGTTDEISSAICFALELVTEKGHTFAEELQKIQCTLQDVGSALATPCSSARESAHLKYTTPKAGPILELEQWIDKY
consensus   81 ......*..***......     .    .  .* ..* ... ... **.....  .*....  .... . ..*..** .

EcPduO     101 MNAVPPVHSFILPGRCEAASRVHFARTVARRAERRLVELTTETIVRNVLLHYINRLSDCLYALARVEDNVAHQNLMIQEI
MxPduO     102 EAELPPMTHFILPGGTQAASALHLARTVCRRAERRTVPLLREGKLPKAVVVYLNRLSDLLFVLARVVN------------
HsMMAB     161 TSQLPPLTAFILFSGGKISSALHFCRAVCRRAERRVVPLVQMGETDANVAKFLNRLSDYLFTLARYAA------------
consensus  161    .**.. ****..  ..*..*..*.*.******.*.*. .. .   .....***** *. ***.

EcPduO     181 TKRYHEANHTPALKERTMPLTFQDLHQLIRSAAMRADELHIPVVISIVDANGTESVTWRMPDALLVSSELAPKKAWTAVA
MxPduO     170 ----HRAS-------------------------------------------VEDVKW--------------------LP
HsMMAB     229 ------------------------------------------------------------------------------M
consensus  241     . .                                                . . .                  .

EcPduO     261 MKTATHKLADTVQPGAPLYGLESHMQGKVVTFGGGFPLWRDGKLLGGLGISGGSVEQDMDIAQSAMAAINVGVNQ
MxPduO     182 AKPSK-------------------------------------------------------------------------
HsMMAB     230 KEGNQEKIYMKNDPSAESEGL-------------------------------------------------------
consensus  321  .    ..     . .   ..
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Fig. S3. Sequence alignment of *M. xanthus* PduO (*Mx*PduO) with its human (*Hs*MMAB) and *E. coli* (*Ec*PduO) equivalents. NCBI accession codes for *E. coli*, *M. xanthus*, and *Homo sapiens* PduO are CAJ87624, ABF90014, and NP_443077, respectively. Residues are shaded black if identical and gray if similar in at least two of the aligned sequences, with residues conserved in all three sequences indicated by an asterisk in the line labeled "consensus." The approximately 185-residue N-terminal domain of the larger *E. coli* PduO is sufficient for ATR activity (1). It is this domain that is similar in sequence to the smaller human PduO [whose ATR activity has been demonstrated in vitro (2)] and to that in *M. xanthus*.

1. Johnson CL, Buszko ML, Bobik TA (2004) Purification and initial characterization of the *Salmonella enterica* PduO ATP:Cob(I)alamin adenosyltransferase. *J Bacteriol* 186:7881–7887.
2. Leal NA, Olteanu H, Banerjee R, Bobik TA (2004) Human ATP:Cob(I)alamin adenosyltransferase and its interaction with methionine synthase reductase. *J Biol Chem* 279:47536–47542.

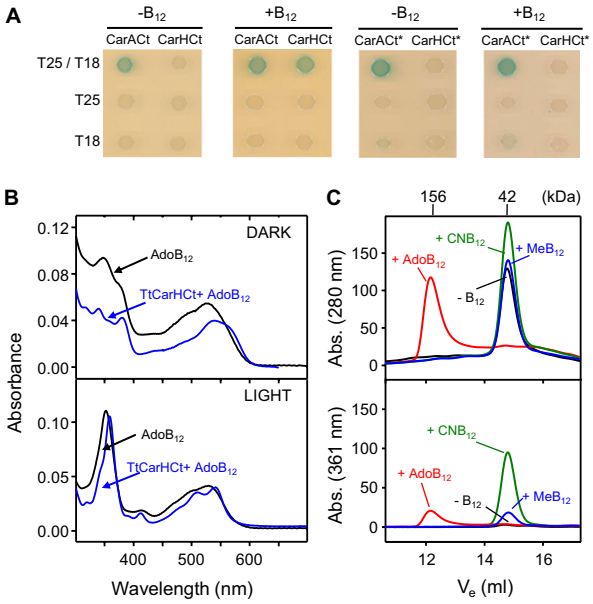

Fig. S4. Analysis of CarACt, CarHCt, and CTt2 interactions using two-hybrid analysis and SEC. (*A*) Two-hybrid analysis in *E. coli* for interactions of CarACt, its H175 to A variant (CarACt*), CarHCt, and its H193 to A variant (CarHCt*). Cells expressing fusions of the indicated C-terminal domain to both T25 and T18, or to just one (negative controls) were spotted on plates containing X-gal and grown in the dark. Interaction, if any, correlates with the intensity of the blue color developed by the spot. (*B*) Visible absorption spectra for TtCarHCt with $AdoB_{12}$ (blue lines) and free $AdoB_{12}$ (black lines) for samples corresponding to the peaks eluted off a Superdex200 analytical column in the dark (*Top*) or after a 5-min exposure to light (*Bottom*). (*C*) Elution profiles off a Superdex200 for 45 μM CTt2 alone (black lines) or with excess $AdoB_{12}$ (red lines), CNB_{12} (green lines), or MeB_{12} (blue lines) tracked by the absorbance (in arbitrary units) at 280 nm (*Top*) or 361 nm (*Bottom*). CTt2 (calculated monomer molecular weight of 33.2 kDa) alone or in the presence of CNB_{12} or MeB_{12} elutes with an M_r of 41.3 ± 0.6 kDa, and as a tetramer (M_r = 156 ± 1 kDa) in the presence of $AdoB_{12}$. M_r for each peak maximum is shown at the top.

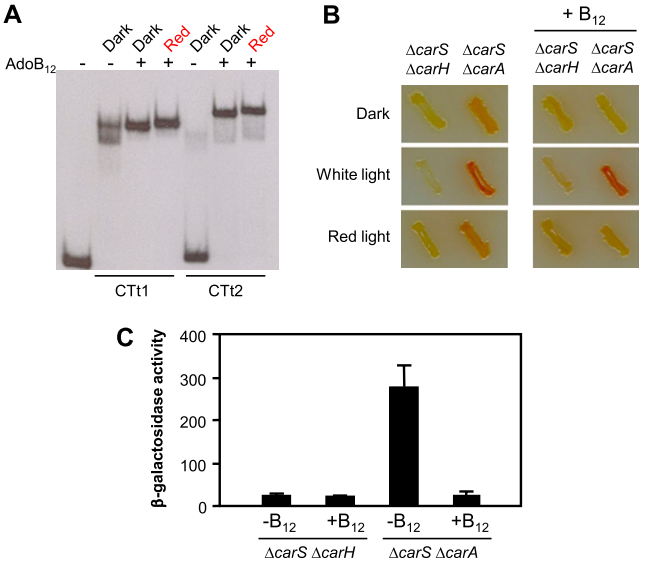

Fig. S5. Red light does not affect $AdoB_{12}$-enhanced DNA binding in vitro, nor relieve B_{12}-dependent CarH-mediated down-regulation of P_B in vivo. (*A*) EMSA with probe CCR130 and 30 nM CTt1 or CTt2 for samples with and without $AdoB_{12}$ (fivefold excess relative to protein) in the dark and for samples with $AdoB_{12}$ that were irradiated with red light. (*B*) Colony color phenotype for the indicated *M. xanthus* strains grown in the dark or under white or red light with and without 1 μM CNB_{12}. (*C*) Reporter P_B-*lacZ* expression (in terms of specific β-gal activity) for cultures of the indicated *M. xanthus* strains grown under red light in the presence or absence of 1 μM CNB_{12}. The mean of three independent measurements and the SEM for the specific β-gal activities are shown.

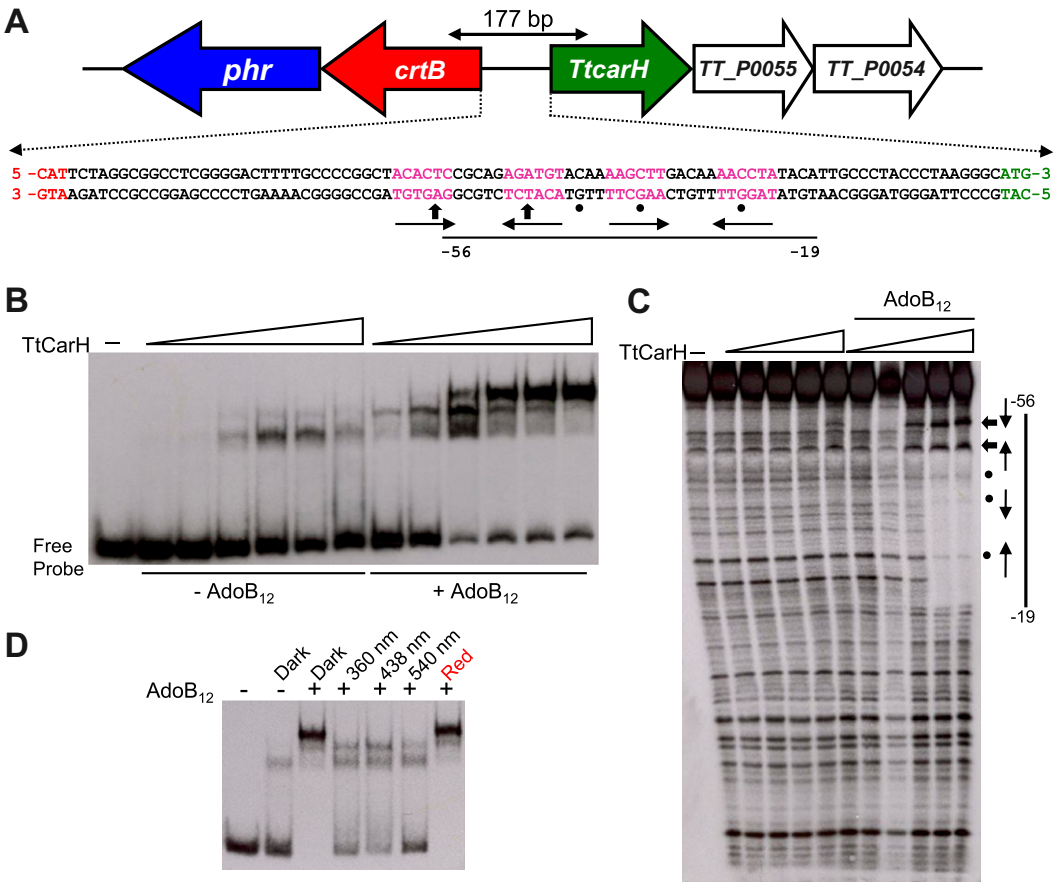

Fig. S6. $AdoB_{12}$ specifically enhances binding of TtCarH to a DNA probe from *T. thermophilus* that contains a CarA/CarH-like operator. (*A*) Genome context of *TtcarH* in *T. thermophilus* megaplasmid pTT27 (NCBI accession no. AE017222). Neighboring gene annotations are as follows: *crtB*, phytoene synthase; *phr*, deoxyribodipyrimidine photolyase; *TT_P0055*, CAP/FNR-type regulatory protein; and *TT_P0054*, NADH-ubiquinone oxidoreductase subunit. The 177-bp probe (double-headed arrow at top) used in DNA-binding assays contains the 92-bp *TtcarH-crtB* intergenic region (sequence shown) with 38 bp upstream and 47 bp downstream segments. The annotated initiator codon for *TtcarH* is in green and that for *crtB* in red. The four segments that resemble the CarA/CarH bipartite operator are in magenta with thin inverted arrows below. The line further below spans the DNase I footprint. Numbers are relative to the translation initiation codon for *TtcarH*. The thick arrows point to hypersensitive sites, and the dots to sites that are not protected. (*B*) EMSA with TtCarH (25, 50, 100, 200, 400, and 900 nM, from left to right) and the 177-bp probe in the dark with and without $AdoB_{12}$. (*C*) DNase I footprint for TtCarH (25, 50, 100, 200, and 400, from left to right) binding with and without $AdoB_{12}$ to the 177-bp probe (5′-labeled in the bottom strand in *A*). (*D*) EMSA with 400 nM TtCarH with and without $AdoB_{12}$ added, and incubated in the dark (35 min) or exposed to the indicated light (5 min) after 30 min incubation in the dark. Leftmost lane shows probe alone. In *B*–*D*, $AdoB_{12}$, when present, is at a fivefold molar excess relative to TtCarH.

Table S1. CarA/CarH-like proteins in bacteria

Bacteria	Taxonomical group	NCBI accession no.
MerR DNA-binding domain + B_{12}-binding domain (CarA/CarH-like protein)		
*Haliangium ochraceum**	δ-Proteobacteria (Myxococcales)	YP_003266651
*M. xanthus**	δ-Proteobacteria (Myxococcales)	CAA79964 (CarA), CAA79965 (CarH)
*Plesiocystis pacifica**	δ-Proteobacteria (Myxococcales)	ZP_01911070
*Sorangium cellulosum**	δ-Proteobacteria (Myxococcales)	YP_001611216
*S. aurantiaca**	δ-Proteobacteria (Myxococcales)	YP_003956654, YP_003956655
Bdellovibrio bacteriovorus	δ-Proteobacteria	NP_967879
Desulfuromonas acetoxidans	δ-Proteobacteria	ZP_01313691
Desulfohalobium retbaense	δ-Proteobacteria	YP_003198393
Geobacter lovleyi	δ-Proteobacteria	YP_001951625
*Meiothermus ruber**	Deinococcus-thermus	YP_003506173
*Meiothermus silvanus**	Deinococcus-thermus	YP_003684883
*Thermus aquaticus**	Deinococcus-thermus	ZP_03497494
*T. thermophilus**	Deinococcus-thermus	YP_145339 (TtCarH)
Truepera radiovictrix	Deinococcus-thermus	YP_003706311
Agrobacterium vitis	α-Proteobacteria	YP_002550011
Hoeflea phototrophica	α-Proteobacteria	ZP_02166227
Acidovorax delafieldii	β-Proteobacteria	ZP_04760938
Acidovorax ebreus	β-Proteobacteria	YP_002553836
Acidovorax sp.	β-Proteobacteria	YP_987184
Azoarcus sp.	β-Proteobacteria	YP_934810
Bordetella avium	β-Proteobacteria	YP_784891
Candidatus accumulibacter	β-Proteobacteria	YP_003169177
Dechloromonas aromatica	β-Proteobacteria	YP_283714
Delftia acidovorans	β-Proteobacteria	YP_001565315
Herminiimonas arsenicoxydans	β-Proteobacteria	CAL62551
Laribacter hongkongensis	β-Proteobacteria	YP_002794311
Leptothrix cholodnii	β-Proteobacteria	YP_001789500
Limnobacter sp.	β-Proteobacteria	ZP_01915313
Methylovorus sp.	β-Proteobacteria	YP_003051081
Methylibium petroleiphilum	β-Proteobacteria	YP_001019435
Polaromonas naphthalenivorans	β-Proteobacteria	YP_981683
Ralstonia eutropha	β-Proteobacteria	YP_293403
Thauera sp.	β-Proteobacteria	YP_002354707
Thiomonas intermedia	β-Proteobacteria	YP_003644171
Thiomonas sp.	β-Proteobacteria	CAZ89507
Methylobacter tundripaludum	γ-Proteobacteria	ZP_07654418
Nitrosococcus halophilus	γ-Proteobacteria	YP_003525843
Magnetococcus sp.	Proteobacteria	YP_867583
Actinosynnema mirum	Actinobacteria	YP_003104187
*Amycolatopsis mediterranei**	Actinobacteria	YP_003766261
Dermacoccus sp.	Actinobacteria	ZP_07704773
Frankia sp. *EuI1c*	Actinobacteria	ZP_06238726
Janibacter sp.	Actinobacteria	ZP_00994632
Micromonospora sp.	Actinobacteria	EEP72734
*Nakamurella multipartita**	Actinobacteria	YP_003199787
*Nocardia farcinica**	Actinobacteria	YP_119275
*Streptomyces ambofaciens**	Actinobacteria	CAJ88224
*Streptomyces avermitilis**	Actinobacteria	BAC68924
*Streptomyces coelicolor**[†]	Actinobacteria	NP_624528 (LitR)
Streptomyces flavogriseus	Actinobacteria	ZP_05801878
Streptomyces ghanaensis	Actinobacteria	ZP_04684938
*Streptomyces lividans**	Actinobacteria	ZP_05528694
Streptomyces pristinaespiralis	Actinobacteria	EDY64715
Streptomyces sp. *C*	Actinobacteria	ZP_07285287
Streptomyces sviceus	Actinobacteria	EFH28311
*Streptosporangium roseum**	Actinobacteria	YP_003337347
*Chitinophaga pinensis**	Bacteroidetes	YP_003125248
*Croceibacter atlanticus**	Bacteroidetes	YP_003714950
*Cytophaga hutchinsonii**	Bacteroidetes	YP_677677, YP_678640
*Dokdonia donghaensis**	Bacteroidetes	ZP_01051271
*Dyadobacter fermentans**	Bacteroidetes	YP_003086417
*Flavobacteria bacterium**	Bacteroidetes	ZP_01202526

Table S1. Cont.

Bacteria	Taxonomical group	NCBI accession no.
*Kordia algicida**	Bacteroidetes	ZP_02163391
*Leeuwenhoekiella blandensis**	Bacteroidetes	ZP_01060805
Microscilla marina	Bacteroidetes	ZP_01688448
*Pedobacter heparinus**	Bacteroidetes	YP_003093970
*Psychroflexus torquis**	Bacteroidetes	ZP_01253496
*Rhodothermus marinus**	Bacteroidetes	ACY48488
*Robiginitalea biformata**	Bacteroidetes	YP_003195366
*Zunongwangia profunda**	Bacteroidetes	YP_003583423
*Chloroherpeton thalassium**	Chlorobi	YP_001996324
Anoxybacillus flavithermus	Firmicutes	YP_002315426
Bacillus cereus sp.	Firmicutes	YP_001374529
Bacillus cellulosilyticus	Firmicutes	ZP_06362586
Bacillus megaterium	Firmicutes	YP_003564800
Bacillus mycoides	Firmicutes	ZP_04156336
Bacillus pseudofirmus	Firmicutes	YP_003427992
Bacillus pseudomycoides	Firmicutes	ZP_04150564
Bacillus selenitireducens	Firmicutes	YP_003700225
Bacillus thuringiensis	Firmicutes	ZP_04144840
Bacillus weihenstephanensis	Firmicutes	YP_001644286
Exiguobacterium sibiricum	Firmicutes	YP_001814285
Exiguobacterium sp.	Firmicutes	YP_002887336
Heliobacterium modesticaldum	Firmicutes	YP_001680059
*Lysinibacillus fusiformis**	Firmicutes	ZP_07050820
Paenibacillus sp.	Firmicutes	YP_003014814
*Chloroflexus aggregans**	Chloroflexi	YP_002462292
*Chloroflexus aurantiacus**	Chloroflexi	YP_001636279
Chloroflexus sp.*	Chloroflexi	YP_002570616
*Herpetosiphon aurantiacus**	Chloroflexi	YP_001545468
Ktedonobacter racemifer	Chloroflexi	ZP_06969864
Oscillochloris trichoides	Chloroflexi	ZP_07686602
*Roseiflexus castenholzii**	Chloroflexi	YP_001431863
Roseiflexus sp.*	Chloroflexi	YP_001276447
Pirellula staleyi	Planctomycetes	ADB17396
Planctomyces maris	Planctomycetes	ZP_01852990
Planctomyces brasiliensis	Planctomycetes	EFQ19218
Rhodopirellula baltica	Planctomycetes	NP_864391
Opitutus terrae	Verrucomicrobia	YP_001817995
Verrucomicrobium spinosum	Verrucomicrobia	ZP_02929072
Candidatus Nitrospira defluvii	Nitrospirales	CBK39973
*Thermobaculum terrenum**	Thermobaculum	YP_003323754
B_{12}-binding domain + sensor hybrid histidine kinase or response regulator		
Planctomyces maris	Planctomycetes	ZP_01857708
Clostridium cellulovorans	Firmicutes	YP_003842171
Moorella thermoacetica	Firmicutes	YP_430949
Cyanothece sp.	Cyanobacteria	YP_002485444, YP_003889199
Taxonomical group	BLAST hits vs. TtCarHCt ($E < 0.001$)	Length (no. residues)
Standalone B_{12}-binding domain proteins		
δ-Proteobacteria[‡]	12	207–239
α-Proteobacteria	12	192–241
Actinobacteria	4	199–227
Firmicutes	33	160–233
Bacteroidetes	2	220, 224

*Gene for CarA/CarH-like protein located in the vicinity of gene encoding a carotenogenic enzyme and/or photolyase in the corresponding bacterial genome.
[†]CarA/CarH like protein implicated in light-induced carotenogenesis (1).
[‡]One of these occurs in *M. xanthus* (NCBI Acc. No. YP_628514).

1. Takano H, Obitsu S, Beppu T, Ueda K (2005) Light-induced carotenogenesis in *Streptomyces coelicolor* A3(2): Identification of an extracytoplasmic function sigma factor that directs photodependent transcription of the carotenoid biosynthesis gene cluster. *J Bacteriol* 187:1825–1832.

Table S2. Strains and plasmids used in this work

Strain or plasmid	Relevant phenotype	Relevant genotype or description	Source/ref.*
M. xanthus			
DK1050	Car^+	WT	1
MR418	Car^- Tc^R $LacZ^I$	*carB::*Tn*5-lac*-132 (transposon insertion [ΩMR401:: Tn*5-lac*])	2
MR844	$Car^{(+)}$	Δ*carA*	2
MR848	Car^+	Δ*carH*	2
MR1716	Car^C	Δ*carA* Δ*carH*	3
MR1728	$Car^{(+)}$ Km^R	Δ*carA* Δ*carH;* P_C*::carH*	3
MR1744	Car^-	Δ*carA carB*::Tn*5-lac*-132	Present study
MR1776	Car^-	Δ*carS*	4
MR1777	$Car^{(+)}$	Δ*carS* Δ*carA*	Present study
MR1778	Car^C	Δ*carS* Δ*carA* Δ*carH*	Present study
MR1780	Car^- Tc^R $LacZ^-$	Δ*carS carB*::Tn*5-lac*-132	Present study
MR1781	Car^- Tc^R $LacZ^{(+)}$	Δ*carS* Δ*carA carB*::Tn*5-lac*-132	Present study
MR1782	Car^- Tc^R $LacZ^c$	Δ*carS* Δ*carA* Δ*carH carB*::Tn*5-lac*-132	Present study
MR1793	Car^-	Δ*carS* Δ*carH*	Present study
MR1794	Car^- Tc^R $LacZ^-$	Δ*carS* Δ*carH carB*::Tn*5-lac*-132	Present study
MR2063	$Car^{(+)}$ Km^R	Δ*carA* Δ*carH;* P_C*::carA(1-79)/carH(94-299)*	Present study
MR2064	$Car^{(+)}$ Km^R	Δ*carA* Δ*carH;* P_C*::carA(1-74)/carH(73-299)*	Present study
MR2071	$Car^{(+)}$ Km^R	Δ*carA* Δ*carH;* P_C*::carA(1-79)/TtcarH(80-285)*	Present study
MR2072	$Car^{(+)}$ Km^R	Δ*carA* Δ*carH;* P_C*::carH(1-93)/TtcarH(80-285)*	Present study
MR2075	Car^C	Δ*carA* Δ*pduO*	Present study
MR2076	Car^- Tet^R $LacZ^C$	Δ*carA* Δ*pduO carB*::Tn*5-lac*-132	Present study
E. coli			
DH5α	—	φ80 *lacZ*ΔM15 Δ*lacU169 recA1 endA1 hsdR17 supE44 thi-1 gyrA relA1*	5
BL21(DE3)	—	F^-*ompT hsdS*$_B$ ($r_B^-m_B^-$) *gal dcm* with DE3, a λ prophage carrying the T7 RNA polymerase gene	Novagen
JM109	—	e14–(McrA–) *recA1 endA1 gyrA96 thi-1 hsdR17* (r_K^- m_K^+) *supE44 relA1* Δ(*lac-proAB*) [F′ *traD36 proAB lacI*^{q}*ZΔM15*]	6
BTH101	—	F^-*cya99I alaD139 galE15 galK16 rpsL* (Str^R) *hsdR2 mcrA1 mcrB1*	7
Plasmids			
pET15b	Km^R	Protein overexpression vector	Novagen
pKT25	Km^R	Vector for C-terminal fusion to the T25 fragment of CyaA	7
pKT25Zip	Km^R	GCN4 zipper positive control for two-hybrid analysis	7
pMR2828	Km^R	Vector with *carH(Nter)* expressed from a constitutive promoter (P_C)	3
pMR2830	Km^R	Vector with *carH* expressed from P_C	3
pMR2838	Km^R	Vector with *carA* expressed from P_C	3
pMR3014	Amp^R	Vector overexpressing His_6-tagged TtCarH	Present study
pMR3184	Km^R Gal^S	Vector with in-frame Δ*carS* allele	4
pMR3334	Km^R	Vector with gene encoding chimera C1: CarA(1-79) + CarH(94-299) expressed from P_C	Present study
pMR3335	Km^R	Contains gene encoding chimera C2: CarA(1-74)+ CarH(73-299) expressed from P_C	Present study
pMR3363	Km^R	Contains gene encoding chimera CTt2: CarH(1-93) + TtCarH(76-285) expressed from P_C	Present study
pMR3370	Km^R	Contains gene encoding chimera CTt1: CarA(1-79) + TtCarH(76-285) expressed from P_C	Present study
pMR3375	Amp^R	pUT18C-*carH(73-299)*	Present study
pMR3376	Km^R	pKT25-*carH(73-299)*	Present study
pMR3381	Amp^R	pUT18C-*carA(75-288)*	Present study
pMR3382	Km^R	pKT25-*carA(75-288)*	Present study
pMR3390	Amp^R	pUT18C-*carA(75-288) (H175A)*	Present study
pMR3391	Km^R	pKT25-*carA(75-288) (H175A)*	Present study
pMR3392	Amp^R	pUT18C-*carH(73-299) (H193A)*	Present study
pMR3393	Km^R	pKT25-*carH(73-299) (H193A)*	Present study
pMR3394	Amp^R	pUT18C-*TtcarH(76-285)*	Present study
pMR3395	Km^R	pKT25-*TtcarH(76-285)*	Present study
pMR3425	Km^R Gal^s	Vector with in-frame Δ*pduO* allele for constructing Δ*pduO* strain	Present study
pMR3451	Amp^R	Vector for overexpressing intein-tagged CarA	Present study
pMR3469	Amp^R	Vector for overexpressing intein-tagged CTt1	Present study
pMR3470	Amp^R	Vector for overexpressing intein-tagged CTt2	Present study

Table S2. Cont.

Strain or plasmid	Relevant phenotype	Relevant genotype or description	Source/ref.*
pMR3471	Amp^R	Vector for overexpressing intein-tagged TtCarHCt	Present study
pMR3516	Amp^R	pUT18C-*TtcarH(76-285) (H177A)*	Present study
pMR3517	Km^R	pKT25-*TtcarH(76-285) (H177A)*	Present study
pMR3522	Amp^R	Vector for overexpressing intein-tagged CarACt	Present study
pMR3536	Amp^R	Vector for overexpressing intein-tagged TtCarHCt (H177A)	Present study
pTYB12	Amp^R	Vector for overexpressing intein-fusion proteins	New England Biolabs
pUT18C	Amp^R	Vector for C-terminal fusions to the T18 fragment of CyaA	7
pUT18CZip	Amp^R	GCN4 zipper positive control for two-hybrid analysis	7

*Strains constructed in this study as described in *SI Materials and Methods*.

Car^+, WT phenotype for light-induced carotenogenesis; Car^-, defective in light-induced carotenogenesis (note: strains with the *carB*, Tn*5-lac*-132 transposon insertion probe are Car^-); Car^C, constitutive phenotype for carotenogenesis; $Car^{(+)}$, WT phenotype for carotenogenesis in the presence of B_{12}. Km^R, kanamycin resistance. Tc^R, tetracycline resistance. P_C, constitutive promoter. $LacZ^I$, light-inducible synthesis of β-galactosidase; $LacZ^C$, constitutive synthesis of β-gal; $LacZ^{(+)}$, WT synthesis of β-gal in presence of B_{12}; $LacZ^-$, low β-gal levels.

1. Ruiz-Vázquez R, Murillo FJ (1984) Abnormal motility and fruiting behavior of *Myxococcus xanthus* bacteriophage-resistant strains induced by a clear-plaque mutant of bacteriophage Mx8. *J Bacteriol* 160:818–821.
2. Cervantes M, Murillo FJ (2002) Role for vitamin B_{12} in light induction of gene expression in the bacterium *Myxococcus xanthus*. *J Bacteriol* 184:2215–2224.
3. Pérez-Marín MC, Padmanabhan S, Polanco MC, Murillo FJ, Elías-Arnanz M (2008) Vitamin B_{12} partners the CarH repressor to downregulate a photoinducible promoter in *Myxococcus xanthus*. *Mol Microbiol* 67:804–819.
4. León E, et al. (2010) A bacterial antirepressor with SH3 domain topology mimics operator DNA in sequestering the repressor DNA recognition helix. *Nucleic Acids Res* 38:5226–5241.
5. Hanahan D (1983) Studies on transformation of *Escherichia coli* with plasmids. *J Mol Biol* 166:557–580.
6. Yanisch-Perron C, Vieira J, Messing J (1985) Improved M13 phage cloning vectors and host strains: Nucleotide sequences of the M13mp18 and pUC19 vectors. *Gene* 33:103–119.
7. Karimova G, Ullmann A, Ladant D (2000) A bacterial two-hybrid system that exploits a cAMP signaling cascade in *Escherichia coli*. *Methods Enzymol* 328:59–73.