SUPPLEMENTAL DATA

A bacterial antirepressor with SH3 domain topology mimics operator DNA in sequestering the repressor DNA recognition helix

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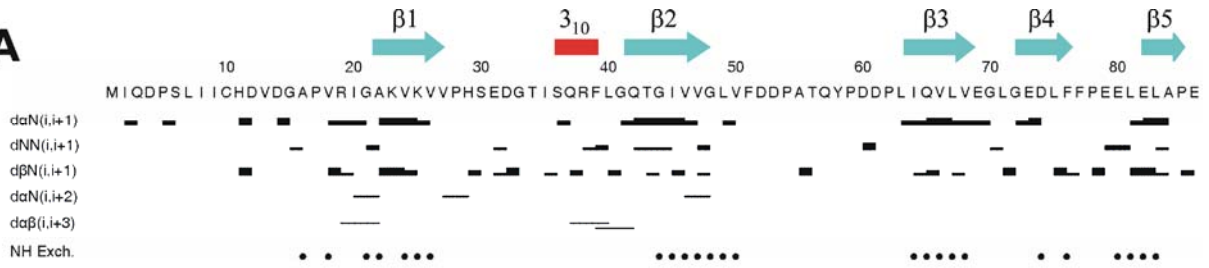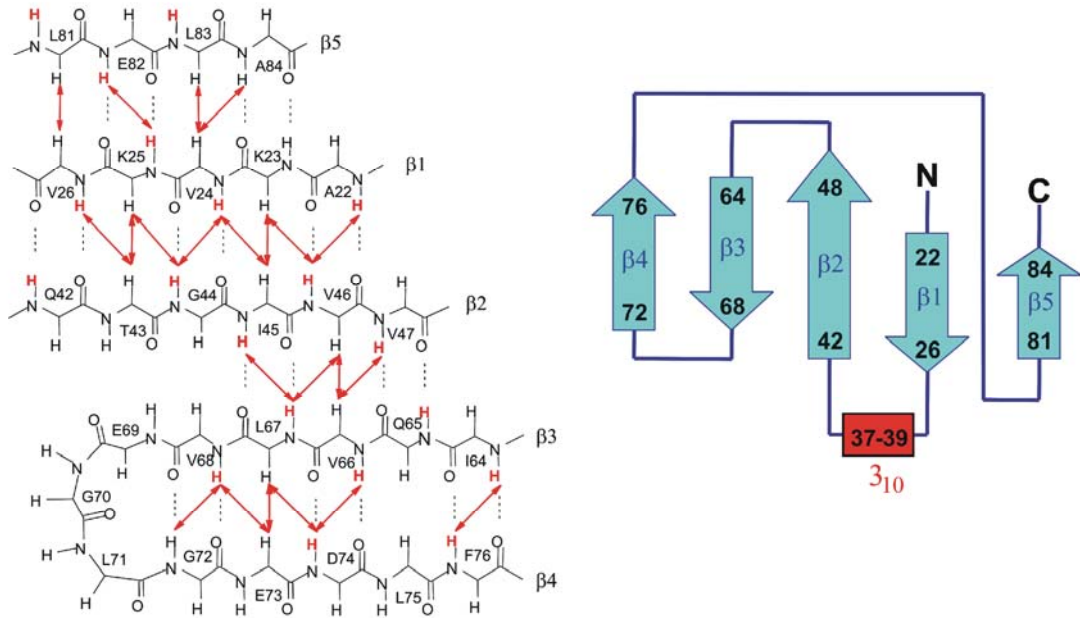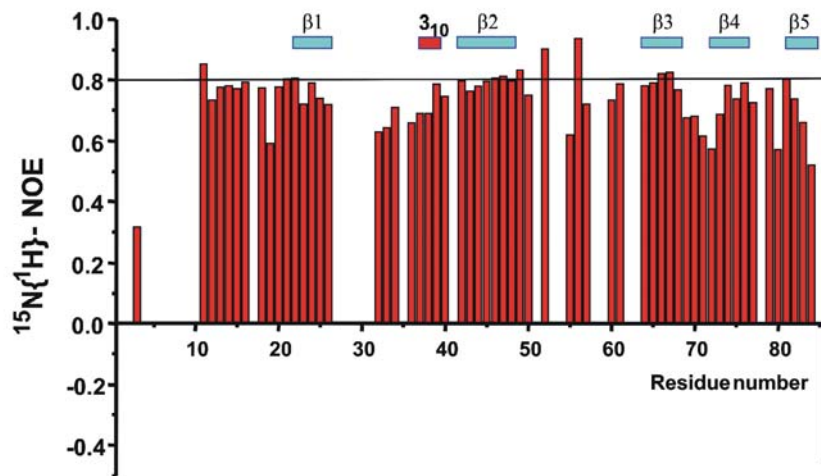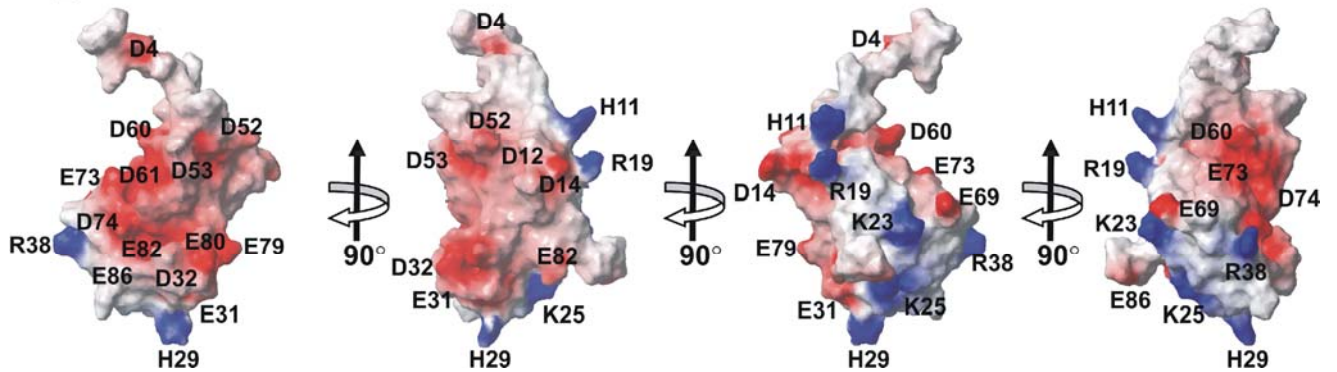
Supplementary Table S1. Strains and plasmids used in this work

Strain or plasmid	Relevant phenotype	Genotype or description	Source or reference
***M. xanthus* strains**			
DK1050	Car$^+$	Wild-type	(1)
MR1776*	Car$^-$	ΔcarS	pMR3184 x DK1050
MR1935*	Car$^+$, Km^R	carS´ (encodes CarS with non-native R-I-L following M1)/ ΔcarS	pMR3233 x MR1776
MR1936*	Car$^-$, Km^R	carS´(D52A/D53A) / ΔcarS	pMR3234 x MR1776
MR1937*	Car$^-$, Km^R	carS´(E79A/E80A) / ΔcarS	pMR3235 x MR1776
MR1938*	Car$^-$, Km^R	carS´(L63A) / ΔcarS	pMR3267 x MR1776
MR1939*	Car$^-$, Km^R	carS´(L75A) / ΔcarS	pMR3264 x MR1776
MR1940*	Car$^-$, Km^R	carS´(F77A) / ΔcarS	pMR3268 x MR1776
Plasmids			
pET15b	Amp^R	Vector for overexpression of H$_6$-tagged protein.	Novagen
pBJ114	Km^R Gal^S	Vector to generate gene deletions/mutations in *M. xanthus*.	(2)
pEG202	Amp^R*HIS3*	Vector for expressing N-terminal fusions to the LexA DNA-binding domain (DBD) under the control of ADH1 promoter for yeast two-hybrid analysis.	(3)
pJG4-5	Amp^R*TRP1*	Vector for expressing N-terminal fusions to the B42 transcriptional activation domain (TAD) under control of the galactose-inducible GAL1 promoter for yeast two-hybrid analysis.	(3)
pSH18-34	Amp^R*URA 3 lacZ*	*lacZ* under the control of a GAL10-8 LexA-GAL promoter region. Reporter for yeast two-hybrid analysis.	(3)
pMAR975	Km^R Gal^S	pBJ114 derivative lacking an *Eco*RI site	(4)
pMR2606	Amp^R*TRP1*	pJG4-5 derivative for expressing CarS fused to B42-TAD	(5)
pMR2609	Amp^R*HIS3*	pEG202 derivative for expressing CarA fused to LexA DBD	(5)
pMR3125	Amp^R	pET15b derivative for overexpression of H$_6$-CarS(D52A/D53A)	This work
pMR3126	Amp^R	pET15b derivative for overexpression of H$_6$-CarH(Nter)(R27A)	This work
pMR3138	Amp^R*TRP1*	pJG4-5 derivative for expressing CarS(E31A/D32A) fused to B42-TAD	This work
pMR3139	Amp^R*TRP1*	pJG4-5 derivative for expressing CarS(D52A/D53A) fused to B42-TAD	This work
pMR3140	Amp^R*TRP1*	pJG4-5 derivative for expressing CarS(D60A/D61A) fused to B42-TAD	This work
pMR3141	Amp^R*TRP1*	pJG4-5 derivative for expressing CarS(E73A/D74A) fused to B42-TAD	This work
pMR3142	Amp^R*TRP1*	pJG4-5 derivative for expressing CarS(E79A/E80A) fused to B42-TAD	This work
pMR3155	Amp^R*TRP1*	pJG4-5 derivative for expressing CarS(D12A/D14A) fused to B42-TAD	This work
pMR3156	Amp^R*TRP1*	pJG4-5 derivative for expressing CarS(E69A) fused to B42-TAD	This work
pMR3179	Amp^R*TRP1*	pJG4-5 derivative for expressing CarS(D4A) fused to B42-TAD	This work
pMR3180	Amp^R*TRP1*	pJG4-5 derivative for expressing CarS(E82A) fused to B42-TAD	This work
pMR3181	Amp^R*TRP1*	pJG4-5 derivative for expressing CarS(E86A) fused to B42-TAD	This work
pMR3184	Km^R Gal^S	pMAR975 with the ΔcarS allele used to generate *carS*-deleted *M. xanthus*	This work
pMR3233	Km^R Gal^S	pMR3184 with *carS´* introduced into the *Eco*RI site.	This work
pMR3234	Km^R Gal^S	pMR3184 with *carS´(D52A/D53A)* introduced into the *Eco*RI site.	This work
pMR3235	Km^R Gal^S	pMR3184 derivative with *carS´(E79A/E80A)* introduced into *Eco*RI site.	This work
pMR3249	Amp^R	pET15b derivative for overexpression of H$_6$-CarS(F77A)	This work
pMR3261	Amp^R*TRP1*	pJG4-5 derivative for expressing CarS(F77A) fused to B42-TAD	This work
pMR3262	Amp^R*TRP1*	pJG4-5 derivative for expressing CarS(L75A) fused to B42-TAD	This work
pMR3263	Amp^R*TRP1*	pJG4-5 derivative for expressing CarS(L63A) fused to B42-TAD	This work
pMR3264	Km^R Gal^S	pMR3184 derivative with *carS´(L75A)* introduced into the *Eco*RI site.	This work
pMR3267	Km^R Gal^S	pMR3184 derivative with *carS´(L63A)* introduced into the *Eco*RI site.	This work
pMR3268	Km^R Gal^S	pMR3184 derivative with *carS´(F77A)* introduced into the *Eco*RI site.	This work

* Generated in this study as described in SI Materials and methods. Car$^+$: wild-type phenotype for light-induced carotenogenesis; Car$^-$: null phenotype for light-induced carotenogenesis.

Supplementary Table S2. NMR data and structural statistics for the final structures of CarS1

Total number of NOE distance constraints	968
Intraresidue (i-j=0)	287
Sequential ($\left|i\text{-}j\right|= 1$)	246
Medium-range ($2\le\left|i\text{-}j\right|\le 1$)	105
Long-range ($\left|i\text{-}j\right|\ge 5$)	330
Dihedral angle constraints (TALOS)	
Total (ϕ,ψ)	141
Pairwise root mean square deviations (Å)	
Backbone atoms (residues 1-86)	3.4 ± 1.0
Backbone atoms (residues 10-86)	0.8 ± 0.1
Heavy atoms (residues 1-86)	3.8 ± 1.0
Heavy atoms (residues 10-86)	1.4 ± 0.1
Maximum constraint violations	
Distance (Å)	0.15 ± 0.05
Dihedral angles (º)	2.3 ± 0.7
Structural quality (Ramachandran plot analysis)	
Residues in the most favourable regions (%)	82.3
Residues in additional allowed regions (%)	15.8
Residues in generously allowed regions (%)	0.4
Residues in disallowed regions (%)	1.5

A

β1   3₁₀   β2   β3   β4   β5

10   20   30   40   50   60   70   80

M I Q D P S L I I C H D V D G A P V R I G A K V K V V P H S E D G T I S Q R F L G Q T G I V V G L V F D D P A T Q Y P D D P L I Q V L V E G L G E D L F F P E E L E L A P E

dαN(i,i+1)
dNN(i,i+1)
dβN(i,i+1)
dαN(i,i+2)
dαβ(i,i+3)
NH Exch.

B

β5
β1
β2
β3
β4

β4 76 / 72
β3 64 / 68
β2 48 / 42
β1 22 / 26
N
β5 84 / 81
C
3₁₀ 37-39

C

$^{15}N\{^{1}H\}$- NOE

β1   3₁₀   β2   β3   β4   β5

Residue number

D

Supplementary Figure S1. (**A**) Summary of NMR data showing sequential and medium range NOEs involving backbone NH and C_αH, and side chain $C_\beta$H protons relative to residue i. The thickness of the bars scales with weak, medium, and strong NOE intensities. Elements of secondary structure deduced from NMR data are indicated with β-strands as blue arrows and the 3_{10}-helix as a red bar. Black dots indicate residues with the slowest amide ^{1}H exchange (NH/ND) with solvent. (**B**) Schematics showing the anti-parallel β-sheet topology in CarS1. On the left, non-sequential NOEs are indicated by the red two-headed arrows. Amide ^{1}H in red are those that exchange slowest with solvent. The arrangement of the anti-parallel β-strands and the 3_{10}-helix is shown on the right with delimiting residues numbered. (**C**) Steady-state heteronuclear ^{15}N{^{1}H}-NOEs for the backbone amides plotted against residue number. Secondary structure elements from the NMR structure are indicated on top as in (**A**). (**D**) Four views at 90° clockwise rotations of the electrostatic surface representation of CarS1showing regions of positive and negative charge, respectively, in blue and red, the color intensity scaling with electrostatic potential, and the charged residues labeled.
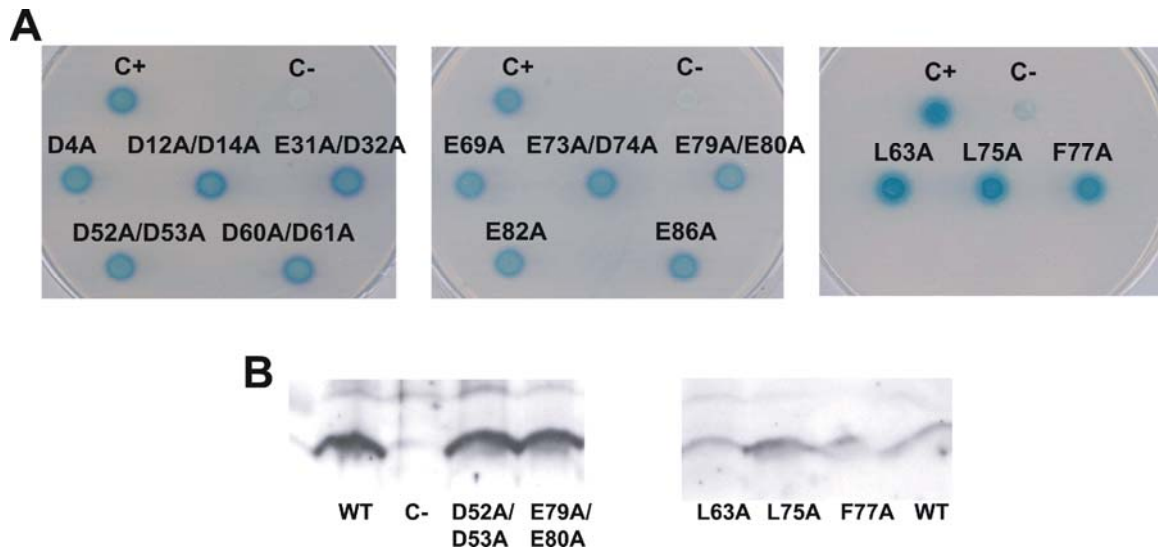
Supplementary Figure S2. Analysis of CarS mutants *in vivo*. (**A**) Yeast two-hybrid analysis of the interactions of each CarS mutant with CarA on galactose plates. C+: LexA-CarA and B42-CarS; C-: LexA-CarA only; the rest express LexA-CarA and the indicated B42-CarS*. (**B**) Western blot analysis using mouse monoclonal anti-CarS antibodies of whole cell extracts of the indicated strains obtained after cell growth in the light. C- is the ΔcarS strain; the others are derived from introducing the indicated allele into the ΔcarS strain.
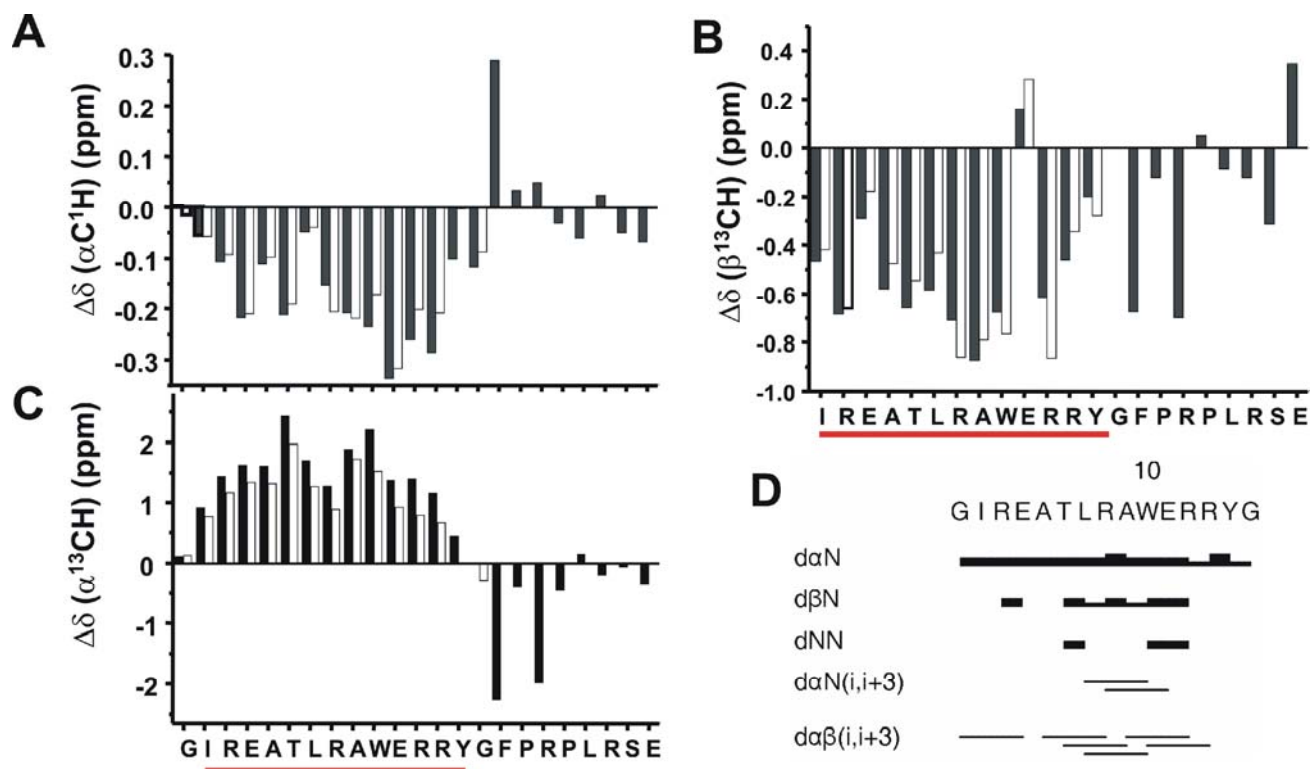
Supplementary Figure S3. NMR analysis of peptides corresponding to α2, the CarA DNA recognition helix. Deviation (Δδ) of the observed chemical shift (δ_{obs}) for peptides P01 (black bars) and P02 (open bars) from the corresponding random coil value (δ_{rc}) shown for the αC^{1}H (**A**), α^{13}CH (**B**), and β^{13}CH (**C**). The helical segment, as inferred from the NMR data, is underlined in red and coincides with α2 in the high resolution NMR structure determined for CarANt. (**D**) Summary of medium and short range NOEs for the shorter P02 peptide. NMR spectra were recorded at 5 °C and at pH 6.5.
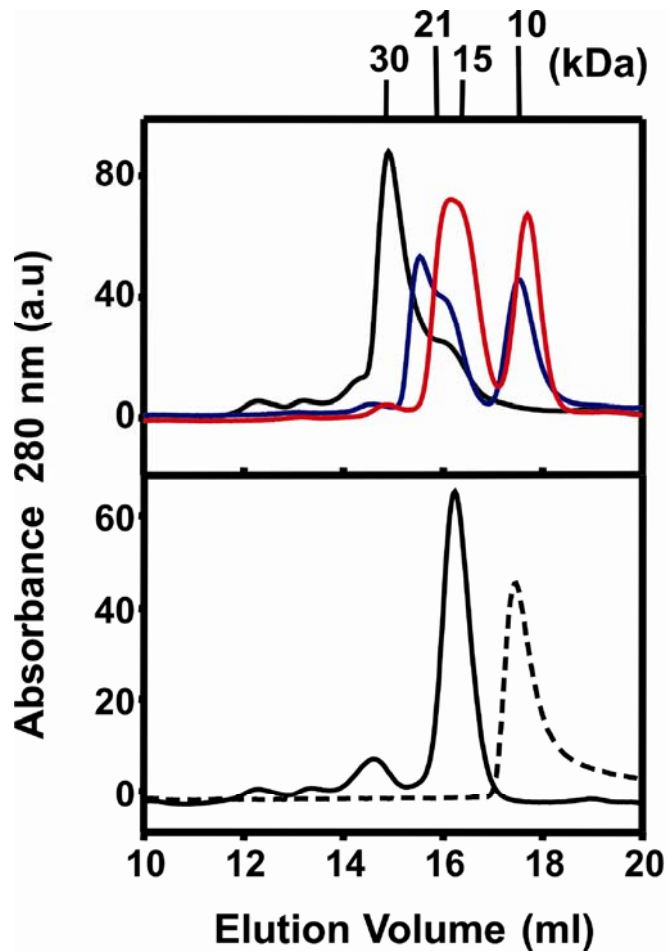
Supplementary Figure S4. Effect of ionic strength on stable CarS-CarANt complex formation. The bottom panel shows the elution profiles for pure CarS (solid curve) or pure CarNt (dashed curve) off a Superdex-200 analytical gel filtration column in buffer containing 150 mM NaCl as described in the main text. The top panel shows the elution profiles for mixtures of CarS and CarNt in buffer with NaCl concentration at 150 mM (black curve), 300 mM (blue curve), and 1 M (red curve). M_r (in kDa) for each peak maximum is indicated. Note that increasing the ionic strength shifts the 30 kDa peak corresponding to the complex towards lower M_r, with a concomitant rise in the intensities of peaks corresponding to free proteins, indicating that stable complex formation is increasingly hindered by higher ionic strength.

A

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CarS       1 MIQDPSLIICHDVDGAPVRIGAKVKVVPHSEDGTISQRFLGQTGIVVGLVFDDPATQYPD
SaCarS     1 MTRDPSLIVTVDVEGAPVRIGEQVRIVSASREDSIDPRFLGCSGIVVALVFDDPWLQYPA
consensus  1  *  ***** .  ** .******* *  *  *   * **** .**** ****** ***

CarS      61 DPLIQVLVEGLGEDLFFPEELELAPEWARNRIAQHRQAVRTGGRSSLERLP
SaCarS    61 DPLIRVRVHGLGEDLFFVRELDGLPARAGAAFRALPPARAC----------
consensus 61 **** * * ********  **  *  *  *             .........
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B

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CarA       1 -MTLRIRTIARMTGIREATLRAWERRYGFPRPLRSEGNNYRVYSREEVEAVRRVARLIQE
SaCarA     1 MSLLRIRTIARLTGIREATLRAWERRHGFPRPHRSE-NNYRAYTREEVENIRRVAKLIEE
consensus  1  . ******* ***************** ****  *** **** ***** .****.** *

CarA      60 EGLSVSEAIAQVKTEPPREQPEAERLRERFWSSVGALEGDEVTRVLDDAQTVMDVEAYCD
SaCarA    60 G-LSVSEAIAQVKALPVGALPAGERLSERFWSAVMGMDTDEAQRVLDEGQASMDVDTYCD
consensus 61  .**********   *   *  ***.*****.*  .   **  ****.  *  ***. ***

CarA     120 GFLLPLLREMGVRLDVAREHLASALIRQRLRQVYDALSPAPAGPRALLACPSGDHHEGGL
SaCarA   119 GFLLPLLREMGGRLDIAREHMASALIRQRLSALMAAEGTRQEGPRVVLACPARDHHEGGL
consensus 121 *********** ***.****.*********  .  *     ***  **** .*******

CarA     180 LVLGIHLKRKGWRVTMLGADTPAAALQGACVQVRPDVVALSFVRARAPEEFASVLEDALR
SaCarA   179 LALGLYLKRRGWRVTMLGADTPAEALRSACAQVRPDLVALSFVRHREPEEMAAVLRECVQ
consensus 181 * **. *** ***************  **.***  ** ***** .**   * **  .. .

CarA     240 ACAPFPVVVGGLGAREHLKAIFSLGAQYAESSEELVAIWNQVRNAQNRP
SaCarA   239 ACSPVLVVVGGAAAREHLKAIFSAGAQYAETASEMMAQWQQARGASNRT
consensus 241 **  * ***** ********** ****** .  * .  * * *  * **
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C

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CarH       1 MAERTYRINIAAELAGVRVELIRAWERRYGVLTPRRTPAGYRAYTDRDVAVLKQLKRLTD
SaCarH     1 MAERTYRIHIAAELSGVSPELIRAWERRYGVPRPLRTPAGYRVYTEQDVALLRRLKQLVG
consensus  1 ******** ***** **   ************ * ******* **  *** * . ** *

CarH      61 EGVAISEAAKLLPQLMEGIEAEVAGRGASQDARPHAETWRESMLAATQAYDQPRVSDVLD
SaCarH    61 EGMSIREAAWASREVEHVQVPPPVLAEGGTSR--TEEWREAVLAAAERYDQTRVSQVLD
consensus 61 **  * *** *    .*   .      .    * . * ***  *** *  ***  ****

CarH     121 EVLAALPPLKAFDEVLAPILLCDVGERWESGTLTVAQEHLVSQMVRARLVSLLHAAPLGR-
SaCarH   119 EVLAALPPLKAFDEVLAPVQRAVGDRWHAGTLTVAQEHLVSQVVRARLVNLLHVAPENLG
consensus 121 ***************** .   .   **  *.***********.****** ***.** .

CarH     180 HRHGVLACFPEEEHEMGLLGAALRLRHLGVRVTLLGQRVPAEDLGRAVLALRPDFVGLST
SaCarH   179 QRHAVLGCFPDEEHEVGLLGAALRLRHAGIRVSLLGQRVPVKDLGHLVGQLRPHLVGLSA
consensus 181  **.**.***.****.***********.*.**.*******  ***  *  *** .****.

CarH     240 VASRSAEDFEDTLTRLRQALPRGLPVWVGGGAAASHQAVCERLAVHVEQGEEDWDRLAGT
SaCarH   239 VVNPGAAAEFEQTLSELMAVLPAQVPLWVGGPAALHHAEVCARWCARVFRPGDDWAVLLA-
consensus 241 *     **  * ** .*  .**   **.****  ** *  **  * * . .. **  *  ..
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Supplementary Figure S5. Sequence alignments of CarS, CarA, and CarH proteins in *M. xanthus* and *S. aurantiaca*. (**A**) Alignment of CarS proteins. Arrows in magenta point to residues important for CarS interaction with CarA in *M. xanthus* identified in this study. (**B**) Alignment of CarA proteins. (**C**) Alignment of CarH proteins. In (**B**) and (**C**) the helix α2 segment is boxed in red. Identical residues are shaded black and have an asterisk in the "consensus" line below. Similar residues are shaded gray. The *S. aurantiaca* protein sequences are based on our annotation of the genome sequence. We encountered errors in the corresponding genome annotation deposited at NCBI and have commented on it elsewhere (see 6).

SUPPLEMENTARY REFERENCES

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5. López-Rubio,J.J., Elías-Arnanz,M., Padmanabhan,S. and Murillo,F.J. (2002) A repressor-antirepressor pair links two loci controlling light-induced carotenogenesis in *Myxococcus xanthus*. *J. Biol Chem.*, **277**, 7262-7270.

6. Pérez-Marín,M.C., Padmanabhan,S., Polanco,M.C., Murillo,F.J. and Elías-Arnanz,M. (2008) Vitamin B_{12} partners the CarH repressor to downregulate a photoinducible promoter in *Myxococcus xanthus*. *Mol. Microbiol.*, **67**, 804-819.