SUPPLEMENTARY INFORMATION

Signaling between two interacting sensor kinases promotes biofilms and colonization by a bacterial symbiont

Allison N. Norsworthy and Karen L. Visick*

Department of Microbiology and Immunology, Loyola University Medical Center

*Corresponding author

E-mail: kvisick@luc.edu

Address for both authors:

2160 S. First Ave, Bldg. 105, Rm 3933

Maywood, IL 60153

(708)216-0869

A

H-box	Fhxxh(S/T/A)H(D/E)h(R/K)TPLxxh
N-box	(D/N)xxxhxxhhxNLhxNAh(F/H/Y)(S/T)
D-box/F-box	hxhxhxDxGxGhxxxxxxxxhFxxF
G-box	GGxGLGLxhhxxhhxxxxGxhxhxxxxxxGxxFxhxh

B

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                                    H-box
SypF (V. fischeri)    ---KSRFLASMSHEIRTPMNAVLGLLAILKDTTLKPNQKELVNTATDSSELLLSIINDIL
ArcB (E. coli)        SRDKTTFISTISHELRTPLNGIVGLSRILLDTELTAEQEKYLKTIHVSAVTLGNIFNDII
VieS (V. cholerae)    -EARNHFLAVVSHELRTPIAAMLGLMEILASRLKNSESQLLLTNAISSAERLKLHVNDIL
EnvZ (E. coli)        ---RTLLMAGVSHDLRTPLTRIR-----LATEMMSEQDGYLAESINKDIEECNAIIEQFI
                         :. ::: :**::***:  :       *     . :.     .   .       .::::

SypF (V. fischeri)    DFSRMEANTFYLENHIFNIHKSLNSVLKTFHPQAQNKQLELSLFIADNVPTYVQGDAHRL
ArcB (E. coli)        DMDKMERRKVQLDNQPVDFTSFLADLENLSALQAQQKGLRFNLEPTLPLPHQVITDGTRL
VieS (V. cholerae)    DFSKIEAQQLQLDIGLYNLTDELGPLLRGFEASAQLKEIEFDVIWSPNSLLLANFDALRF
EnvZ (E. coli)        DYLRTG---QEMPMEMADLNAVLGEVIA--AESGYEREIETALYPG---SIEVKMHPLSI
                      *  :       :     ::   *  :      ..  : :.  :         .  .   :
                      N-box                                    D-box/F-box
SypF (V. fischeri)    RQILLNLVGNSLKFTDDGQVQILVNAEEHEGRIQLHCSVQDSGIGIQQEQLEYLFDEFTM
ArcB (E. coli)        RQILWNLISNAVKFTQQGQVTVRVRYDEGD---MLHFEVEDSGIGIPQDELDKIFAMYYQ
VieS (V. cholerae)    NQIVTNLLSNAIKFTDQGRVVFKIDVAPEM----LTIVVEDTGCGMTQTQIESLFVPFAQ
EnvZ (E. coli)        KRAVANMVVNAARYGN-GWIKVSSGTEPNR----AWFQVEDDGPGIAPEQRKHLFQPFVR
                      .: : *:: *: :: : * : .              *:* * *:   : . :*  :
                                                G-Box
SypF (V. fischeri)    ADNSFS-RTHEGSGLGLAICQRLVHMMDGTITVNSQYGLGSEFSFNIQLDKATTKE----
ArcB (E. coli)        VKDSHGGKPATGTGIGLAISRRLAKNMGGDITVTSEQGKGSTFTLTIH------------
VieS (V. cholerae)    ADSTIT-RRFGGTGLGMSIVANLIELMNGKIEVKSEFEQGTQIQVNL-------------
EnvZ (E. coli)        GDSART---ISGTGLGLAIVQRIVDNHNGMLELGTSERGGLSIRAWLPVPVTRAQGTTKE
                       ..:       *:*:*:::  .: .  .* : : :.   *  :   :
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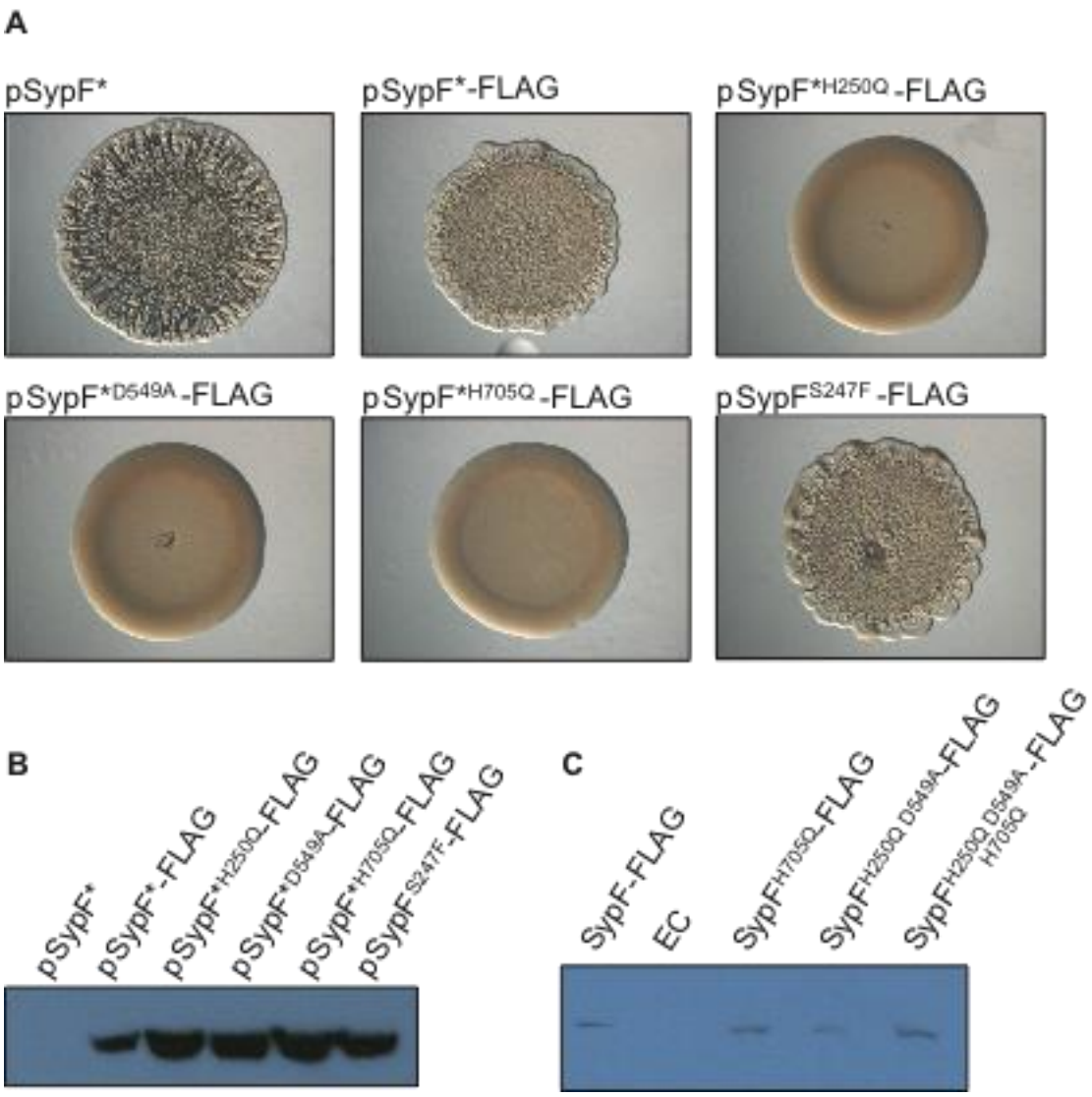
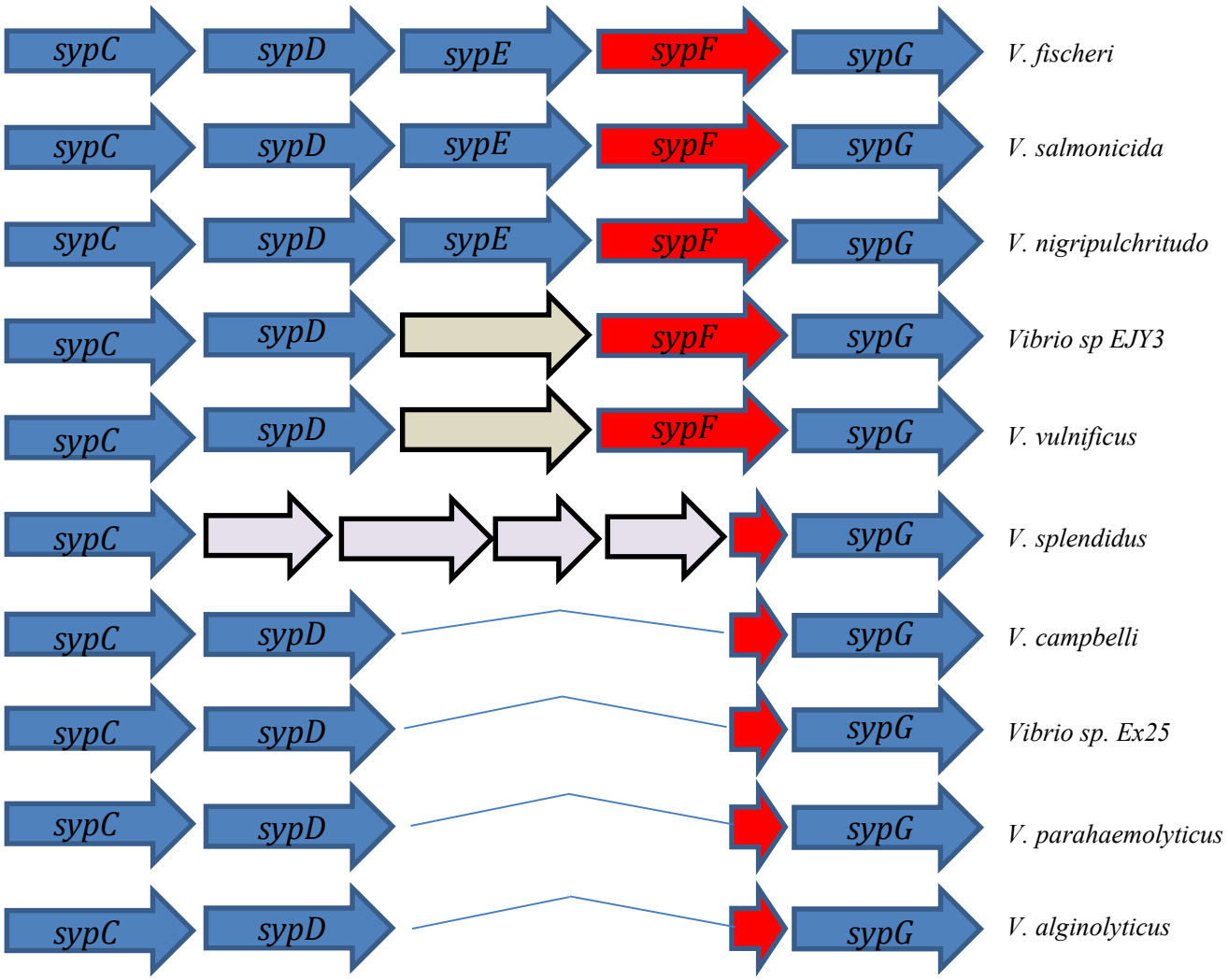
Supplementary Figure S1. Sequence alignment of SypF with known, functional histidine kinases. (A) Homology boxes in HisKA and HATPase_c domains (Grebe and Stock, 1999). The HisKA region contains the site of autophosphorylation within the H-box, and the HATPase_c domain contains the N, D, F, and G-boxes, which bind ATP and/or metal cofactors. (B) Sequence alignment of HisKA and HATPase_c domains from SypF and known functional SKs. Sequences were obtained from the following bacterial strains: *V. fischeri* ES114, *E. coli* MG1655, and *Vibrio cholerae* AC50, and were aligned using the online software, ClustalW, http://embnet.vital-it.ch/software/ClustalW.html. * represents an identical amino acid; : represents a highly conserved amino acid; = represents a moderately conserved amino acid.



Supplementary Figure S2. Wrinkled Colony phenotype and/or expression of SypF*-FLAG and SypF-FLAG variants.

(A) Wrinkled colony assay of wild-type (ES114) cells overproducing SypF variants from a plasmid. Plasmids are as indicated: pSypF* (pCLD29); pSypF*-FLAG (pANN70); pSypF*^{H250Q}-FLAG (pANN71); pSypF*^{D549A}-FLAG (pANN72); pSypF*^{H705Q}-FLAG (pANN76); pSypF^{S247F}-FLAG (pANN73). Cells were spotted on an agar plate and colony morphologies were assessed after 40 hours. (B) Western blot analysis of untagged SypF* and FLAG-tagged SypF proteins from strains used in Supp. 1A. (C) Western blot analysis of FLAG-tagged SypF proteins encoded in single copy from the chromosome of a *sypF* deletion strain (See Fig 5A). EC: empty cassette. Strains are as follows: SypF-FLAG (KV6659); EC (KV6921); SypF^{H705Q}-FLAG (KV7085); SypF$^{H250Q\ D549A}$-FLAG (KV7154); SypF$^{H250Q\ D549A\ H705Q}$-FLAG (KV7155).



Supplementary Figure S3. The genomic region around *sypF* and *sypF*-like HPt-encoding genes in *Vibrio* species. A subset of Vibrio genomes that contain *syp* genes are depicted (Altschul *et al.*, 1997, Altschul *et al.*, 2005). The organisms containing *sypF* or *sypF*-like genes are as follows. *Aliivibrio salmonicida* LFI1238 (VSAL_II0307)(Holland *et al.*, 1997), *Vibrio nigripulchritudo* (VIBNI_A1485)(Goudenege *et al.*, 2013), Vibrio sp. EJY3 (VEJY3_08720)(Roh *et al.*, 2012), *Vibrio vulnificus* YJ016 (VV1628)(Chen *et al.*, 2003), *V. splendidus* LGP32 (VS_1526), *Vibrio campbellii* ATCC BAA-1116 (VIBHAR_02229)(Wang *et al.*, 2013), Vibrio sp. Ex25 (VEA_003532), *Vibrio parahaemolyticus* RMID 2210644 (VP1472)(Makino *et al.*, 2003), and *Vibrio alginolyticus* NBRC 15630 = ATCC 17749 (VAL01S_15_00550). For EJY3 and *V. vulnificus*, the gene in the position of *sypE* encodes a phosphonate ABC transporter substrate-binding protein. For *V. splendidus*, *sypD* and *sypE* are lacking, and four other genes are present between the *sypC*-like gene and the HPt-encoding gene. For the last four, the blue line indicates the absence of *sypE* and the 5' end of *sypF*. Arrows depicting genes are not to scale.

Supplementary Table 1. Plasmids used in this study

Name	*Description*	*Relevant Primers*	*Source or Reference*
pANN17	pKV363 + 3.8 kb sequences flanking *sypE sypF*	1219, 519, 1249, 1375	This study
pANN20	pEVS107 + P_{lac}-*sypF*-FLAG	1609, 1563	This study
pANN21	pEVS107 + P_{lac}-*sypF*^{D549A}-FLAG	1609, 1563	This study
pANN24	pEVS107 + P_{lac}-*sypF*^{H250Q}-FLAG	1609, 1563	This study
pANN34	pEVS107 + P_{lac}-*sypG*-FLAG*	1609 1438	This study
pANN45	pEVS107 + P_{lac}-*sypF*^{H705Q}-FLAG	1795, 1793, 1796, 1794	This study
pANN46	pEVS107 + P_{lac}-*sypF*$^{H705Q\ D549A\ H705Q}$-FLAG	1795, 1793, 1796, 1794	This study
pANN48	pMAL-c5x producing SypF amino acids 95-766	1828, 1829	This study
pANN49	pMAL-c5x producing SypG-REC amino acids 1-118	1809, 1810	This study
pANN50	pARM47[1] + P_{lac}-*sypF*-HPt-FLAG	1902, 1796	This study
pANN52	pANN34 + P_{sypA}-*lacZ*	N/A	This study
pANN58	pARM47[1] + P_{lac}-*sypF*-HPt^{H705Q}-FLAG	1902, 1796	This study
pANN59	pJMO8 + P_{sypA}-*lacZ*	1860, 1861	This study
pANN61	pKV69 + *sypF**^{D549A}	1295	This study
pANN62	pKV69 + *sypF**^{H705Q}	1569	This study
pANN65	pEVS107 + P_{lac}-*sypF*$^{H705Q\ D549A}$-FLAG	1795, 1796	This study
pANN69	pCLD29[2] + *rscS-sypF* chimera-FLAG	1899, 1900, 1901, 1882	This study
pANN70	pCLD29[1] + *sypF**-FLAG	1881, 1882	This study
pANN71	pCLD29[1] + *sypF**^{H705Q}-FLAG	1881, 1786, 1785, 1882	This study
pANN72	pCLD29[1] + *sypF**^{D549A}-FLAG	1295	This study
pANN73	pCLD29[1] + *sypF*^{S247F}-FLAG	1881, 1784, 1783, 1882	This study
pANN74	pMAL-c5x producing SypF* amino acids 95-766	1828, 1829	This study
pANN76	pCLD29[1] + *sypF**^{H705Q}-FLAG	1881, 1793, 1794, 1882	This study
pANN77	pARM47[1] *rscS-sypF* chimera	1908, 1907	This study
pANN78	pARM47[1] *rscS*	1908, 1909	This study
pARM7	pKV282 + *rscS*	N/A	(Morris *et al.*, 2011)
pARM47	pEVS107 + P_{lac} *sypE*	N/A	(Morris *et al.*, 2011)
pARM141	pGEX-5X-1 + *sypE*	N/A	(Morris & Visick, 2013)
pCLD29	pKV69 + *sypF**	N/A	(Darnell *et al.*, 2008)
pCLD54	pKV69 + *sypF*	N/A	(Darnell *et al.*, 2008)
pEVS104	Conjugal helper plasmid (*tra trb*)	N/A	(Stabb & Ruby, 2002)
pEVS107	Mini-Tn*7* delivery plasmid, OriR6K, mob	N/A	(McCann *et al.*, 2003)
pKV282	Vector, Tet^R	N/A	(Morris *et al.*, 2011)
pJET1.2	Commercial cloning vector, Ap^R	N/A	Fermentas
pJMO8	Suicide vector with sequences flanking the Tn*7* site	N/A	(Ondrey & Visick, 2014)
pKPQ17	pKV363 + 1 kb sequences flanking *sypF*	910, 1160, 1249, 271	This study
pKV69	Vector; Cm^R, Tet^R	N/A	(Visick & Skoufos, 2001)
pKV363	Suicide plasmid	N/A	(Shibata *et al.*, 2012)
pKV456	pKV363 + 1.5 kb sequences flanking *rscS*	1494, 1495, 1496, 1497	This study
pMAL-c5X	Commercial MBP tag protein expression vector; Ap^R	N/A	New England Biolabs
pUX-BF13	Transposase expressing vector	N/A	(Bao *et al.*, 1991)

[1] restriction enzymes were used to remove the original *sypE* sequence but maintain P_{lacZ} to drive expression of inserted DNA sequences

[2] the original *sypF** sequence was removed from pCLD29 using restriction enzymes before the insertion of indicated DNA sequences

Supplementary Table 2. Primers used in this study.

Primers

Name	*Sequence (5' - 3')*
271	CTCGGCGCATACTTCTTTAC
519	GGGTGGTGTTACTCGCTAC
910	GTGGTGTAATCATGGCCCATACTCTATTACCACAA
1160	TAGGCGGCCGCACTTAGTATGGATGCACTGAATAATTGAGATACC
1219	TAGGCGGCCGCACTTAGTATGTGTGGGCTTTGTATCTGAAAAAAG
1249	CATACTAAGTGCGGCCGCCTAAAACAAGGTTTCTCAAAATAAAAG
1295	(P)GACCTTATTTTCATGGCTATATCTATGCCTGAAATGGATGGCATGACGGC
1375	TCATCATTCCGATTCTTCATAG
1438	AAAAAGGTACCTTATTTATCATCATCATCTTTATAATCTTCCGATTCTTCATAGGCTTCCCA
1494	TACTGACGTATCCGTGTTGC
1495	GGCCGATGCTAAAGATTCAG
1496	TAGGCGGCCGCACTTAGTATGAATGATTGTGATAAGGCTATAACG
1497	CATACTAAGTGCGGCCGCCTAAAGTATGAAACACAATAAACTTCG
1563	ACCCGGGTTATTTATCATCATCATCTTTATAATCTTGAGAAACCTTGTTTATTTC
1569	(P)GCATTAGAGTTTGAAGCGCAAACATTAGGAAGCAGTGCATTAACG
1609	AACTAGTGGCACGACAGGTTTCCCGAC
1783	GTAAAAGTCGATTTTTAGCTTTCATGAGTCACG
1784	GGGGTTCGTATTTCGTGACTCATGAAAGCTAAAAA
1785	CAATATGACCTTATTTTCATGGCTATATCTATGCC
1786	CATCCATTTCAGGCATAGATATAGCCATGAAAATAAG
1793	AATGCACTGCTTCCTAATGTTTGCGCTTCAAACTC
1794	ATGCATTAGAGTTTGAAGCGCAAACATTAGGAAGC
1795	GATCTACTAGTGGCCAGGTACCGGCACGACAGGTTTCCCGAC
1796	CCAGTCTAGTTCTAGAGGGCCCTTATTTATCATCATCATCTTTATAATC
1809	TCACATATGTCCATGGGCGGCCGCATGCTACAGAAAGTATTATTAG
1810	CAGGGAATTCGGATCCGTCGACTAGGTGGTTAGCAATGGATG
1828	TCACATATGTCCATGGGCGGCCGCATGACATTTCGACTTAAAACG
1829	CAGGGAATTCGGATCCGTCGACTTCTTTTATTTTGAGAAACC
1860	AACCATACTAAGTGCGGCCGCCTCTTAAGTCGATTCTCATTC
1861	GAGAGACAATATAGGCGGCCGCCATAATGGATTTCCTTACGC
1881	GCTTGCATGCCTGCAGGTCGACCATTATTGCTGTTAATTGAG
1882	CGAGCTCGGTACCCGGGGATCCTTATTTATCATCATCATCTTTAT
1899	GCTTGCATGCCTGCAGGTCGACGAATTACTCCCCTAATTACG
1900	TAGCTCATTATCCATTGCATCATCTGAAAGTTTATATTT
1901	CTTTCAGATGATGCAATGGATAATGAGCTATTATTAGTA
1902	GATTACGCCAAGCTTGCATGCAAGGAGCTAACTATGGATAATGAGCTATTATTAG
1907	CCAGTCTAGTTCTAGAGGGCCCTTATTTTGAGAAACCTTGTTTA
1908	GATTACGCCAAGCTTGCATGCGAATTACTCCCCTAATTACGAAC
1909	CCAGTCTAGTTCTAGAGGGCCCCGAAGTTTATTGTGTTTCATAC

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