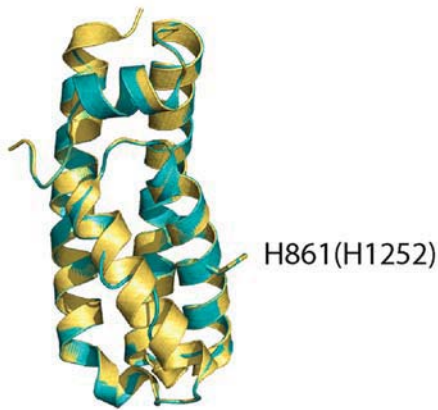
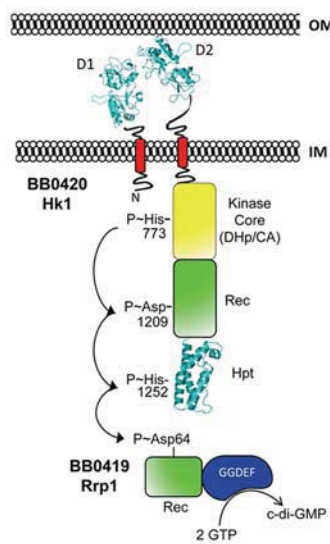


A

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Bburg Hk1  X K E L N L S T V S Y S R L C R G L V D P F I S I N I I D I Z E R A F D E S D D S L I T K D I S N S I T S G A L S N M R S S E L T E D F Q K R I R T S K D S I S R L K R M T F P V E . D D L F P Q L T S D T K R N I T L P E S R I V S E N K L
Shewanella Hk T Q L N H R S N C L A L E W E K M L D S L P E T V R I Q T A L G Q N D G A I T M L S T I H R K H G A I S L C F . . . . . D I G V P T Z Q R L E C Q R I E R S A I R K . E Q T F V S D L E R P S I L L D L L V R F L L Q L L V R F S A V K Q V Q S
Bord BvgS . L T Q N D K A L I R Q L L R E F V I R T N R A D V D Q L K L H Q Q A D P F X I V S D M A N R L A G D A R E . . . . . V D G A K A M I D T V L A L E K R A Q Q Q A G P S P E I D D G E V R T L A A Q S A A L E K T Q L R A W L .
Ecoli ArcB . . . E L V G F K L I R T D L L A V F E R K M W P G Y V S V L E S N L T A Q D R K G V I V G E Q H R K I K G A A G . . . . . S I V G L R M L I Q L G Q Q I Q S P D L . F A H E D N I G D W I R E M K R R W R N D V E V L R A W V L .
Ecoli BvgS . N T A N D C Q L M Q E T L M T P Q R E T H R D L P A A F Q L L F A L G D S R T T H Q C T I H R I H G A A N . . . . . T L N G Q A I N I H Q Q L E I T P V . D D S K P F E L Q L N S I R H M I A R I D Q E I A L E P F C .
Ecoli BarA . . . A A G K T D L A R D M L Q M L Z D F I Z F E V R N K V E E Q L V G E X F E G L V D L I H K L N G S C G . . . . . T S G V P R M L N L C Q L I R Q Q L R . S G T E R E D L E P D L L L D R M D N V A R R A S . . . . .
Psys LemA . . . A A G K A D L A A D M L A M L L A S L D A D R E A I R V A R A N Q D V H A L I E R I R H R L N G A I R E . . . . . Y C G V P Q L R S A C Q R A E T L L K . Q N A P . H T R E L L N D E A I I R L E R A R A W M A .
Xanth RptC . A L G M G E E F E R Q P V R Q C L D D A Q N C T V G D I J E R D G T C S V W R Q L R E S A H A L E R G V A S . . . . . S I G L A Q V I A S I G G E L M R M A D . . . . . W Q L Q A E W R L R L L S T L E R Q L K A G R D A L D A L R V L .

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B**C**

Supplemental Figure 1. Identification of an Hpt domain within Hk1 and predicted phosphorelay scheme between Hk1 and Rrp1. **A.** Multiple sequence alignment between Hk1 putative histidine phosphotransfer domain (Hpt) domain and six prototypical hybrid histidine kinases. Asterisks are used to indicate histidine, glycine, and glutamic acid residues that are highly conserved within a broad range of Hpt domain (SMART Accession Number SM00073). Abbreviations: Bburg, *B. burgdorferi*; Shewanella, *Shewanella* sp. W3-18-1; Ecoli, *Escherichia coli*; Bord, *Bordetella pertussis*; Psys, *Pseudomonas syringae*; Xanth, *Xanthomonas campestris*. **B.** Modeling of Hk1 Hpt domain. The structure of the *B. burgdorferi* Hk1 Hpt domain (residues 1297-1415) was predicted using Swiss-Model and superimposed onto the experimentally-determined structure for the corresponding domain from a *Shewanella* sp. W3-18-1 hybrid histidine kinase (PDB 3MYF) using PyMol. The position of the predicted active site histidine within the *Shewanella* and *B. burgdorferi* (in parentheses) Hpt domains is indicated. **C.** Based on the phosphotransfer schemes of characterized TCS, binding of a ligand(s) by the Hk1 periplasmic domains D1 and/or D2 allosterically activates the cytoplasmic Dhp/CA CA domain, which in turn catalyzes the ATP-dependent autophosphorylation of His773 within the DHp domain. The phosphoryl group is predicted to be transferred first to Asp1209 in the Hk1 receiver (REC), then to His1252 within the C-terminal Hpt domain and, finally, to Asp64 residue within the Rrp1 REC domain. Once activated, Rrp1 catalyzes the synthesis of c-di-GMP from two GTP nucleotides.