The HAMP Linker in Histidine Kinase Dimeric Receptors Is Critical for Symmetric Transmembrane Signal Transduction*

Received for publication, January 29, 2004, and in revised form, July 26, 2004
Published, JBC Papers in Press, August 16, 2004, DOI 10.1074/jbc.M401024200

Yan Zhu and Masayori Inouye‡
From the Department of Biochemistry, Robert Wood Johnson Medical School, Piscataway, New Jersey 08854

The HAMP linker, a common structural element between a sensor and a transmitter module in various sensor proteins, plays an essential role in signal transduction. Here, by in vivo complementation experiments with Tar-EnvZ hybrid receptor mutants in which the HAMP linker forms a heterodimer with Tar and EnvZ-type subunits, we found that mutations at one linker only affect the function of EnvZ in the same subunit. However, the same mutations affect the EnvZ function of both subunits when only a Tar or EnvZ-type HAMP linker is used. These results suggest that intersubunit interactions in the HAMP linker normally mediate signal transduction through both subunits in a sensor dimer, whereas the signal is asymmetrically transduced through the linker in a heterodimer. This is the first demonstration that two HAMP linkers in a sensor dimer are functionally coupled for normal signal transduction; however, this functional coupling can be reduced when the HAMP linkers lose their symmetric nature.

The HAMP linker is widely found in histidine kinases, chemoreceptors, bacterial nucleotidyl cyclases, and phosphatases (1–3). Although the primary sequence homology among predicted HAMP linkers is low, they all share a similar helix-turn-helix fold based on secondary structure prediction. A cysteine-scanning study on the Tar linker supports this model of HAMP linker structure (4).

Genetic analysis found a number of mutations clustered in the HAMP linker of various sensor proteins, which usually lead to biased signaling modes (5–9). This suggests that the HAMP linker plays an active role in signal transduction rather than being just a simple structural element connecting the sensor and transmitter modules of sensor proteins. However, the exact function of the HAMP linker on signal transduction remains to be determined.

Functional hybrid histidine kinases have been constructed by fusing the sensor module of chemoreceptors (also called methyl-accepting chemotaxis proteins) to the transmitter module of histidine kinase Enz using the HAMP linker from either protein. In Taz1, the sensor module of chemoreceptor Tar is fused to the transmitter module of Enz using the Tar linker (10). In Trz, the sensor module of chemoreceptor Trg is fused to the transmitter module of Enz using the Trg linker (11). In Tez1A1 and Tez1PQ, the sensor module of Tar is fused to the transmitter module of EnvZ using the EnvZ linker with one Ala insertion at the N-terminal end and the EnvZ linker with a Pro to Gln mutation at position 185 (the position number is based on EnvZ sequence), respectively (12). A number of functional hybrid histidine kinases have also been constructed by fusing the sensor module of one histidine kinase with the transmitter module of another histidine kinase at the HAMP linker region (NarQ-NarX, NarX-NarQ, and NarX-CpxA fusions) or by replacing the HAMP linker of one histidine kinase (NarX) with the HAMP linker of another histidine kinase (CpxA) (13). In addition, functional hybrid chemoreceptors have been constructed in which Tar-Tsr (14), Taph-Tar (15), and Trg-Tar (16) fusions are made using the Tsr, Tar, and Trg linkers, respectively, and an Aer-Tsr fusion is made using the Aer linker (17). The ligand responsiveness of all these hybrid sensor proteins indicates that a common mechanism may be shared for signal transduction. It also implies that the HAMP linkers of different sensor proteins function in a similar manner for signal propagation. Furthermore, specific interactions within the HAMP linker seem to be critical for the linker function, as several Tar-EnvZ (12, 18), NarX-NarQ (13), and NarX-CpxA (13) hybrid sensor proteins carrying a hybrid HAMP linker exhibit non-regulatable phenotypes in vivo.

Cumulative results have suggested that an intrasubunit signal transduction mechanism is adapted by sensor proteins for transmembrane signaling (19–25). However, it is not clear how the intrasubunit signaling across the membrane is interpreted in the cytoplasmic domain of sensor proteins and what the function is of the HAMP linker in this signal propagation.

In the present report, we carried out in vivo complementation experiments using Tar-EnvZ hybrid receptor mutants, which carry the Tar linker or EnvZ linker in combination with the autophosphorylation site mutation (H243V) or a G2 box mutation (G405A). As shown in Fig. 1A, in this dimer only the active center (active center 2) that was formed between domain A from the G405A mutant and domain B from the H243V mutant is active. Furthermore, we examined the effects of mutations at one of the receptor heterodimer linkers or homodimer linkers on the activities of the two active centers in the EnvZ transmitter dimer. Through these analyses we concluded that an intrasubunit asymmetric signal transduction could occur through the HAMP linker in a receptor heterodimer. However, for native histidine kinases or receptor proteins such as methyl-accepting chemotaxis proteins, intersubunit interactions between the two linkers normally mediate symmetric signal transduction through both subunits in a dimer.

EXPERIMENTAL PROCEDURES

Strains and Plasmids—Strain RU1012 (MC4100 ara-/pompC::Kmr) (10) was used for complementation assay. pYY045 (Tez1H1, CmR) carrying the H243V mutation in the envZ gene (26) was digested by NdeI and BamHI. The released fragments were subcloned into Tez1A1, Tez1PQ, and Tez1 (12), replacing the original NdeI/BamHI fragments to construct Tez1A1H1, Tez1PQH1, and.

* This work was supported by National Institutes of Health Grant GM19043. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1754 solely to indicate this fact.

‡ To whom correspondence should be addressed. Tel.: 732-235-4115; Fax: 732-235-4559; E-mail: inouye@rwja.umdnj.edu.
Tez1H1 with an Amp’ antibiotic marker, respectively. These plasmids were then digested by XbaI and BamHI, and the released fragments were subcloned back into pYY0405, replacing the original XbaI/BamHI fragments for the construction of Tez1A1H1, Tez1PQH1, and Tez1H1 with a Cmr antibiotic marker, respectively.

By site-directed mutagenesis, Tez1A1, Tez1PQ, and Tez1 carrying the G405A mutation in the EnvZ transmitter module were constructed and named Tez1A1(G405A), Tez1PQ(G405A), and Tez1(G405A) (all with the Amp’ antibiotic marker), respectively. In addition, the L189D and F220D mutations were introduced independently into Tez1A1H1, Tez1PQH1, and Tez1H1 (all with the Cmr antibiotic marker) or Tez1A1(G405A), Tez1PQ(G405A), and Tez1(G405A) (all with the Amp’ antibiotic marker) by site-directed mutagenesis. Also, the A231V mutation was introduced into Taz1H1 (pYY0405) (with the Cmr antibiotic marker) and Taz1(G405A) (with the Amp’ antibiotic marker) (27) by site-directed mutagenesis to construct Taz1(A231V/H1) and Taz1(A231V/G405A), respectively. A diagram showing the composition of each receptor variants is shown in Fig. 1B.

**β-Galactosidase Assay**—Each pair of complementing hybrid sensor protein constructs was transformed sequentially into RU1012 cells. β-galactosidase activities of RU1012 cells transformed with various hybrid sensor constructs were determined as previously described (28). For each set of data presented in each figure, we picked three independent colonies for the β-galactosidase assay. The error bars reflect the standard deviations of measurements on the three independent samples. The complementation assay for all the combinations presented in one figure was carried out in one experiment. Each experiment was repeated at least twice, and the data shown in the figure represent one of these experiments. As the absolute value of miller units varied in each experiment but the ratios were kept at a similar level, the ratio rather than the numeric value was used as the basis for defining the phenotypes of different variants. The phenotypes were scored as “aspartate-regulatable” if the ratio of β-galactosidase activities (miller units) with and without aspartate treatment is reproducibly larger than two.

**Preparation of the Membrane Fraction**—Membrane fractions containing different Taz1, Tez1A1, or Tez1PQ variants were prepared as described previously (18). The protein amounts were determined by Bio-Rad protein assay. Equal amounts of total membrane proteins (5 μg) were used for Western blot analysis.
**HAMP Linker Is Essential for Symmetric Signal Transduction**

**RESULTS**

**Signal Transduction through the Hetero-HAMP Linkers**—

Taz1H1, a Taz1 mutant carrying an autophosphorylation site mutation, H243V (termed H1), and Taz1(G405A), a Taz1 mutant harboring a G2 box mutation, G405A, were unable to respond to the Taz1 ligand, aspartate, to activate ompC-lacZ expression in RU1012 cells (26, 27). However, when they were co-expressed as the result of heterodimer formation, one functional active center of EnvZ (Fig. 1A, active center 2) could be formed and enabled the RU1012 cells to respond to aspartate, exhibiting an ompC-lacZ aspartate-regulatable phenotype (Fig. 2, column 1). A similar result was previously observed in the complementation experiment between Taz1H1 and Taz1N6 or Taz1G1 (28).

To examine the role of the HAMP linker in each subunit of a sensor dimer in signal transduction, we carried out a complementation assay using Taz1, Tez1A1, and Tez1IPQ receptor mutants harboring the H1 or the G405A mutation. Combinations of these receptor mutants would result in sensor proteins with only one functional active center of EnvZ in which the HAMP linker forms a heterodimer with the Tar and EnvZ-type subunits or forms a homodimer with either the Tar or EnvZ-type subunit. Note that all Taz1, Tez1A1, and Tez1IPQ homodimers exhibit an aspartate-regulatable phenotype in RU1012 cells (10, 12, 26).

Co-expression of Taz1H1 with Tez1A1(G405A) or Tez1IPQ(G405A) in RU1012 cells resulted in an ompC-lacZ constitutive-on phenotype (Fig. 2, columns 2 and 3, respectively), whereas co-expression of Taz1(G405A) with Tez1A1H1 or Tez1PQH1 in RU1012 cells led to an ompC-lacZ aspartate-regulatable phenotype (Fig. 2, columns 4 and 5, respectively). The results of both sets of heterodimer complementation experiments indicate that having different HAMP linkers in the Taz/Tez heterodimer does not interfere with the dimer formation of the downstream EnvZ transmitter module. Importantly, these results also suggest that the different HAMP linkers in the Taz/Tez heterodimer lead to asymmetric activities for the two active centers of EnvZ. The aspartate-regulatable complementation between Taz1(G405A) and Tez1A1H1 or Tez1PQH1 further implies that the HAMP linker of a single subunit of the sensor dimer is sufficient for signal transduction.

**The Effect of HAMP Linker Mutations on Signal Transduction**—Next we examined the effect of mutations in the Tar or EnvZ linker on signal transduction of the linker heterodimers.

It has been shown that the A231V mutation in the Tar linker of Taz1 resulted in an ompC-lacZ constitutive-off phenotype in RU1012 cells (18). We introduced this A231V mutation into Taz1H1 and Taz1(G405A) to construct Taz1(A231V/H1) and Taz1(A231V/G405A) and then examined the effect of this mutation on the complementation between Taz1H1 and Tez1A1(G405A) (Fig. 3A, column 2) or between Taz1H1 and Tez1PQ(G405A) (Fig. 3A, column 3) as well as between Taz1(G405A) and Tez1A1H1 (Fig. 3A, column 4) or between Taz1(G405A) and Tez1PQH1 (Fig. 3A, column 5). We found that RU1012 cells co-expressing Taz1(A231V/H1) with Tez1A1(G405A) or Tez1PQ(G405A) retained ompC-lacZ constitutive-on phenotypes, whereas RU1012 cells co-expressing Taz1(A231V/G405A) with Tez1A1H1 or Tez1PQH1 exhibited ompC-lacZ constitutive-off phenotypes. Note that without the A231V mutation, co-expression of Taz1(A231V/G405A) with Tez1A1H1 (Fig. 2, column 4) or Tez1PQH1 (Fig. 2, column 5) resulted in an ompC-lacZ aspartate-regulatable phenotype.

Therefore, within the Taz/Tez heterodimers, the A231V mutation in the Tar linker of Taz1 affected the function of the EnvZ transmitter domain only when the same subunit (the Taz1 subunit) contributed the conserved autophosphorylation site His residue for the active center. On the other hand, in the case of the Taz1 linker homodimer, co-expression of Taz1(A231V/H1) with Taz1(G405A) (Fig. 3A, column 1) and Taz1(A231V/G405A) with Taz1H1 (Fig. 3A, column 6) both led to ompC-lacZ constitutive-off phenotypes, indicating that the A231V mutation affects both active centers of EnvZ, no matter in which of the two subunits the mutation is present. By Western blot, we examined the membrane fraction of RU1012 cells co-expressed with Taz1, Tez1A1, or Tez1IPQ variants. As shown in Fig. 3B, although we could not distinguish individual receptor variants, both proteins appeared to be expressed in the co-expression system. The membrane fractions of RU1012 cells co-expressed Taz1(A231V/H1) with Tez1A1H1 (lane 4) or Tez1PQH1 (lane 5), and Taz1(A231V/G405A) with Tez1(G405A) (lane 2) or Tez1PQ(G405A) (lane 3) contained more receptor proteins compared with those of RU1012 cells expressing Taz1(A231V/H1) alone (lane 1). Note that all the non-functional sensor proteins were expressed and localized in the membrane at a similar level when individually expressed (data not shown). In addition, using the membrane fractions isolated in lanes 2–5, the kinase activity of EnvZ could be recovered (data not shown).

We also introduced the L189D or F220D mutation, known to cause an ompC-lacZ constitutive-on phenotype in EnvZ (5), into the Tez1A1(G405A) or Tez1A1H1 linker (the residue numbers are based on the EnvZ sequence) and re-examined the complementation assay between Tez1A1(G405A) and Taz1H1 or between Tez1A1H1 and Taz1(G405A). Consistent with the observation shown in Fig. 3A, aspartate-regulatable phenotypes were retained when the mutations (L189D in Fig. 3C and F220D in Fig. 3D) were added to Tez1A1H1 forming a heterodimer with Taz1(G405A) (Fig. 3C, column 2, and D, column 2, respectively). These results indicate that the mutations in the Tez1A1 linker have no effect on the function of the EnvZ transmitter module when the Taz1 subunit contributes the conserved His residue for the active center. Similar results were obtained by co-expression of Tez1IPQ (L189D/H1 or F220D/H1) with Taz1(G405A) or Tez1IPQ (L189D/G405A or F220D/G405A) with Taz1H1 (data not shown). Also, in the case of the linker homodimer, both mutations in the Tez1A1 (L189D in Fig. 3C, columns 3 and 4, and F220D in Fig. 3D, columns 3 and 4) or in the Tez1IPQ (data not shown) caused ompC-lacZ constitutive-on phenotypes, no matter in which of the two subunits the mutations were present. The effect of the linker mutations on the phenotypes of the linker heterodimers supports the notion that the input signal across the membrane can...
be propagated only through one of the HAMP linkers in a linker heterodimer. There is a possibility that the EnvZ linker interacts directly with the intrasubunit kinase domain to modulate its activity, leading to the observation that mutations at one linker affect the function of EnvZ only in the same subunit. However, this is highly unlikely because the linker mutations affected the phenotypes of the linker homodimers, suggesting that intersubunit interactions in the linker region are responsible for mediating signal transduction through both linkers in a dimer. The fact that both the Tar and EnvZ linkers behave in a similar way in the complementation assays indicates that the structurally conserved HAMP linkers have a similar function in signal transduction.

Complementation between Tez1 and Taz1/Tez1A1/Tez1PQ—Unlike Tez1A1 and Tez1PQ, Tez1 in which the sensor module of Tar is connected to the transmitter module of EnvZ through the EnvZ linker could not respond to aspartate and resulted in an $ompC$-lacZ constitutive-off phenotype in RU1012 cells (12). It has been suggested that the phase of the EnvZ linker in Tez1 might be changed in respect to the transmembrane domain of Tar, in turn locking the activities of the EnvZ transmitter module in a lower kinase/phosphatase ratio and resulting in the $ompC$-lacZ constitutive-off phenotype. Therefore, it is interesting to test how the EnvZ linker in Tez1 modulates the two active centers of EnvZ in complementation experiments of Tez1 with Taz1, Tez1A1, or Tez1PQ.

![Complementation between Tez1 and Taz1/Tez1A1/Tez1PQ](image)

As shown in Fig. 4A, co-expressing Tez1(G405A) with Tez1H1 resulted in an $ompC$-lacZ constitutive-off phenotype in RU1012 cells. On the other hand, co-expressing Tez1H1 with Taz1(G405A) (Fig. 4A, column 2), Tez1A1(G405A) (column 3), or Tez1PQ(G405A) (column 4) resulted in aspartate-regulatable phenotypes for the first two complementations and a constitutive-on phenotype for the last complementation. At the same time, RU1012 cells harboring Taz1(G405A) with Taz1H1 (Fig. 4A, column 5), Tez1A1H1 (column 6), or Tez1PQH1 (column 7) exhibited a constitutive-on, an aspartate-regulatable, or a constitutive-off phenotype, respectively. It is interesting that although the Tez1 linker is non-functional in the Tez1 homodimer, the signal input could still be transduced through the Taz1 linker in the Taz1/Tez1 heterodimer. It is also intriguing that co-expressing Tez1 with Tez1A1 and co-expressing Tez1 with Tez1PQ in RU1012 cells resulted in different phenotypes. The exact mechanisms for these differences remain to be determined; however, these complementation data indicate that the Tez1 linker and the HAMP linker in Taz1, Tez1A1, or Tez1PQ asymmetrically modulate the two active centers in the downstream EnvZ transmitter module in a linker heterodimer.

Next, we examined the effect of the F220D mutation on the complementation of Tez1 with Taz1, Tez1A1, or Tez1PQ. It has been shown that the F220D mutation in Tez1 resulted in an $ompC$-lacZ constitutive-on phenotype in RU1012 cells (12). In the case of Tez1 linker homodimers, RU1012 cells co-expressing Tez1(F220D/H1) with Tez1(G405A) or Tez1(F220D/G405A) with Tez1H1 exhibited $ompC$-lacZ constitutive-on phenotypes (Fig. 4B, columns 1 and 5, respectively). However, in Taz1/Tez1 heterodimers, co-expressing Tez1(F220D/H1) with Taz1(G405A) (Fig.
Fig. 4. Signal transduction through the heterodimers formed between
Tez1 and Taz1/Tez1A1/Tez1PQ. A, complementation between Tez1 and Taz1/
Tez1A1/Tez1PQ. B, the effect of the F220D mutation in the Tez1 linker on
signal transduction. The F220D mutation was introduced into Tez1H1 and Tez1-
(G405A) by site-directed mutagenesis. Each pair of indicated complementing hy-
brid sensor protein constructs was trans-
formed sequentially into RU1012 cells. $\beta$-Galactosidase activities of RU1012 cells
were measured in the presence (black bar) or absence (hatched bar) of 5 mM aspartate
in the growth medium. Error bars indicate standard deviations.

In this study, we tested the in vivo signaling function of
HAMP linker heterodimers and homodimers. Complementa-
tion experiments were carried out using Tar-EnvZ hybrid re-
ceptor mutants in which the HAMP linker forms a heterodimer
with Tar and EnvZ type subunits. The results indicate that
the two linkers in a linker heterodimer appear to take two inde-
pendent conformations in respect to each other and thus differ-
entially modulate the two active centers in the EnvZ trans-

mutter module to result in different in vivo phenotypes. RU1012 cells co-expressing Taz1(G405A) with Taz1A1H1 or co-expressing Taz1(G405A) with Taz1PQH1 could respond to aspartate, leading to ompC-lacZ aspartate-regulatable phenotypes. The A231V mutation in the Tar linker blocked signal transduction and the L189D/F220D in the Taz1A1 or the Taz1PQ linker had no effect on the aspartate-regulatable phenotypes. This further suggests that in the Taz/Tez heterodimer, signal transduction through the HAMP linker is mediated by one single subunit, the Taz1 subunit, which contributes the conserved His residue for the active center.

It is intriguing that, like the co-expression of Taz1(G405A) with Taz1A1H1 or Taz1PQH1, the co-expression of Taz1H1 with Taz1A1(G405A) led to an ompC-lacZ aspartate-regulatable phenotype. Incorporation of the F220D mutation in the Taz1H1 linker also had no effect on this aspartate-regulatable phenotype. Note that homodimers of Taz1, Taz1A1, and Taz1PQ are able to respond to aspartate, whereas homodimers of Tez1 are non-functional, exhibiting an ompC-lacZ constitutive-off phenotype in RU1012 cells (10, 12, 26). These results support the conclusion that the HAMP linkers in a heterodimer could independently modulate the activities of two downstream active centers of the receptor transmitter module.

Interestingly, in the case of linker homodimers, complementing Taz1H1 with Taz1(G405A), Taz1A1H1 with Taz1A1(G405A), and Taz1PQH1 with Taz1PQ(G405A) led to ompC-lacZ aspartate-regulatable phenotypes in RU1012 cells. Incorporation of the HAMP linker mutations in either subunit within these linker homodimers blocked signal transduction, no matter which subunit in a dimer contributed the conserved His residue for the active center. These results indicate that when the two linkers form a homodimer, linker mutations cause a dominant effect on signal transduction, in contrast to the effect of the linker mutations on the linker heterodimers. The complementation experiments between hybrid receptors harboring the EnvZ linker (Tez1) and its variants (Taz1A1 and Taz1PQ) or between Taz1A1 and Taz1PQ further suggest that the signal input cannot be asymmetrically transduced when the HAMP linkers in a dimer are derived from the same origin. Possible intersubunit interactions between the two linkers may prevent the individual linker from functioning independently.

Taken together, our results provide evidence for the first time that in a linker heterodimer, signal input from the periplasmic sensor module can be asymmetrically transduced through the linker domain to the transmitter module. However, in normal signal transduction by histidine kinases or receptor proteins, such as methyl-accepting chemotaxis proteins as seen in a linker homodimer, signal transduction cannot occur asymmetrically through the linker domain because of the interactions between the linkers of both subunits in a dimer.

It has been suggested that an intrasubunit asymmetric signal transduction mechanism is adapted by sensor proteins, particularly by Tar, for transmembrane signaling (19–25). A piston, rotation, tilt, or combination of those movements may be generated by ligand binding and further transduced across the membrane (19, 29–33). It also has been implied that such an intrasubunit asymmetric signal transduction mechanism may apply to signal propagation through the cytoplasmic portion of sensor protein dimers (20–22). In Taz1, when the signal is asymmetrically generated through only one of the two ligand-binding pockets in the Tar receptor domain, the signal-generating subunit appears to be responsible for signal transduction (20). Also, Tar dimers with only one functional cytoplasmic domain are able to respond to aspartate, suggesting that the signal input can be propagated through a single subunit in a dimer (21, 22). However, although these approaches indicate that the intrasubunit signaling is sufficient for the receptor function, it cannot be ruled out that the asymmetric signal across the membrane may be able to be transduced through both subunits in a sensor protein dimer. In Taz1, aspartate-binding site mutations that block signal generation in one subunit caused high basal level ompC-lacZ expression in RU1012 cells for the active center of EnvZ containing the conserved His residue on the same subunit. Therefore, it was not feasible to monitor signal transduction in the non-signal generating subunit. Furthermore, the Tar heterodimer with only one cytoplasmic domain makes it impossible to examine the possible intersubunit signal transduction mechanism. Here, our results demonstrate that although intrasubunit asymmetric signal transduction could occur in a linker heterodimer, it could not be maintained in a linker homodimer because the two HAMP linkers are functionally coupled to
convert the asymmetric signal input across the membrane into a symmetric signal affecting both subunits in the cytoplasmic transmitter module.

Different models have been proposed for the function of the HAMP linkers as to how they mediate proper signal propagation (3, 12). The wide distribution of the HAMP linkers in different sensor proteins, together with their structural similarities, suggests that they may share common mechanisms for signal transduction. Our previous study (12) has suggested that specific interactions between the two predicted helices in the HAMP linker are critical for the linker function, as the two helices have to be derived from the same protein for construction of a functional Tar-EnvZ hybrid receptor. A similar conclusion was drawn from a recent report characterizing a set of NarX-NarQ, NarX-CpxA, and NarQ-CpxA hybrid receptors (13). Our present data further support the previous proposal that the HAMP linker adapts a four-helix bundle structure in a dimer receptor as shown in Fig. 1A (12). The specific interactions between the two helices within one HAMP linker subunit allow a proper signal transduction, whereas the intersubunit interactions within the four-helix bundle enable the signal input to be further propagated in both HAMP linker subunits. An important and unique aspect of histidine kinases and other receptor proteins, such as methyl-accepting chemotaxis proteins, is their need to function as a dimer. The ability of the HAMP linker to modulate the function of the two downstream active centers in a dimer by converting an asymmetric input signal into a symmetric signal may present a unique mechanism for signal transduction in these sensor proteins.

Acknowledgments—We thank M. Ikura, S. Phadtare, J. Zhang, and T. Yoshida for critical reading of the manuscript.

REFERENCES
1. Heinz, D. W., Essen, L. O., and Williams, R. L. (1998) J. Mol. Biol. 275, 635–650
2. Aravind, L., and Ponting, C. P. (1999) FEMS Microbiol. Lett. 176, 111–116
3. Williams, S. B., and Stewart, V. (1999) Mol. Microbiol. 33, 1093–1102
4. Butler, S. L., and Falke, J. J. (1998) Biochemistry 37, 10746–10756
5. Park, H., and Inouye, M. (1997) J. Bacteriol. 179, 4382–4390
6. Harlock, S. L., Rampersaud, A., Yang, W. P., and Inouye, M. (1993) J. Bacteriol. 175, 1956–1960
7. Taylor, R. R., Hall, M. N., and Silhavy, T. J. (1983) J. Mol. Biol. 166, 273–282
8. Waukau, J., and Forst, S. (1992) J. Bacteriol. 174, 1522–1527
9. Tokishita, S., Kojima, A., and Mizuno, T. (1992) J. Biochem. (Tokyo) 111, 707–713
10. Utsumi, R., Brissette, R. E., Rampersaud, A., Forst, S. A., Oosawa, K., and Inouye, M. (1989) Science 245, 1246–1249
11. Baumgartner, J. W., Kim, C., Brissette, R. E., Inouye, M., Park, C., and Hazelbauer, G. L. (1994) J. Bacteriol. 176, 1157–1163
12. Zhu, Y., and Inouye, M. (2003) J. Biol. Chem. 278, 22812–22819
13. Appleman, J. A., Chen, L. L., and Stewart, V. (2003) J. Bacteriol. 185, 4872–4882
14. Kristich, C. J., Conley, M. P., Boyd, A., Berg, H. C., and Simon, M. I. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 1326–1330
15. Weerasuriya, S., Schneider, B. M., and Manson, M. D. (1998) J. Bacteriol. 180, 914–920
16. Feng, X., Baumgartner, J. W., and Hazelbauer, G. L. (1997) J. Bacteriol. 179, 6714–6720
17. Kristitch, C. J., Glekas, G. D., and Ordal, G. W. (2003) Mol. Microbiol. 47, 1353–1366
18. Jin, T., and Inouye, M. (1993) J. Mol. Biol. 232, 484–492
19. Chervitz, S. A., and Falke, J. J. (1995) J. Biol. Chem. 270, 24043–24053
20. Yang, Y., Park, H., and Inouye, M. (1993) J. Mol. Biol. 232, 483–498
21. Gardina, P. J., and Manson, M. D. (1996) Science 274, 425–426
22. Tatuno, I., Homma, M., Oosawa, K., and Kawagishi, I. (1996) Science 274, 425–426
23. Mulligan, D. L., and Kosmidis, D. E., Jr. (1991) Science 254, 1651–1654
24. Zhang, Y., Gardina, P. J., Kuebler, A. S., Kang, H. S., Christopher, J. A., and Manson, M. D. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 939–944
25. Yeh, J. J., Biemann, H. P., Pandit, J., Kosmidis, D. E., and Kim, S. H. (1993) J. Biol. Chem. 268, 9787–9792
26. Yang, Y., and Inouye, M. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 11057–11061
27. Zhu, Y., and Inouye, M. (2002) Mol. Microbiol. 45, 653–663
28. Yang, Y., and Inouye, M. (1993) J. Mol. Biol. 211, 335–342
29. Chervitz, S. A., and Falke, J. J. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 2545–2550
30. Ottemann, K. M., Xiao, W., Shin, Y. K., and Koshland, D. E., Jr. (1999) Science 285, 1751–1754
31. Maruyama, I. N., Mikawa, Y. G., and Maruyama, H. I. (1995) J. Mol. Biol. 253, 539–546
32. Kim, S. H., Prive, G. G., Yeh, J., Scott, W. G., and Milburn, M. V. (1992) Cold Spring Harbor Symp. Quant. Biol. 57, 17–24
33. Stoddard, B. L., Biemann, H. P., and Koshland, D. E., Jr. (1992) Cold Spring Harbor Symp. Quant. Biol. 57, 1–15
The HAMP Linker in Histidine Kinase Dimeric Receptors Is Critical for Symmetric Transmembrane Signal Transduction
Yan Zhu and Masayori Inouye

J. Biol. Chem. 2004, 279:48152-48158.
doi: 10.1074/jbc.M401024200 originally published online August 16, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M401024200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 33 references, 21 of which can be accessed free at http://www.jbc.org/content/279/46/48152.full.html#ref-list-1