Bacteria possess a signal transduction system, referred to as a two-component system, for adaptation to external stimuli. Each two-component system consists of a sensor protein-histidine kinase (HK) and a response regulator (RR), together forming a signal transduction pathway via histidyl-aspartyl phospho-relay. A total of 30 sensor HKs, including as yet uncharacterized putative HKs (BaeS, BasS, CreC, CusS, HydH, RstB, YedV, and YfhK), and a total of 34 RRs, including putative RRs (BaeR, BasR, CreB, CusR, HydG, RstA, YedW, YfhA, YgeK, and YhjB), have been suggested to exist in *Escherichia coli*. We have purified the carboxyl-terminal catalytic domain of 27 sensor HKs and the full-length protein of all 34 RRs to apparent homogeneity. Self-phosphorylation in vitro was detected for 25 HKs. The rate of self-phosphorylation differed among HKs, whereas the level of phosphorylation was generally co-related with the phosphorylation rate. However, the phosphorylation level was low for ArcB, HydH, NarQ, and NtrB even though the reaction rate was fast, whereas the level was high for the slow phosphorylation species BasS, CheA, and CreC. By using the phosphorylated HKs, we examined trans-phosphorylation in vitro of RRs for all possible combinations. Trans-phosphorylation of presumed cognate RRs by HKs was detected, for the first time, for eight pairs, BaeS-BaeR, BasS-BasR, CreC-CreB, CusS-CusR, HydH-HydG, RstB-RstA, YedV-YedW, and YfhK-YfhA. All trans-phosphorylation took place within less than 1/2 min, but the stability of phosphorylated RRs differed, indicating the involvement of de-phosphorylation control. In addition to the trans-phosphorylation between the cognate pairs, we detected trans-phosphorylation between about 3% of non-cognate HK-RR pairs, raising the possibility that the cross-talk in signal transduction takes place between two-component systems.

The two-component system (TCS) is the signal transduction pathway widely employed by prokaryotes to eukaryotes. Typically, TCS is composed of a sensor that monitors an external signal(s) and a response regulator that controls gene expression or other physiological activities such as chemotaxis (1). In bacteria, TCS is the major system of signal transduction but not in Archaea and eukaryotes (2). Most of the sensors of bacterial TCS are membrane-associated histidine kinase (HK). The sensor phosphorolyses its own conserved His residue in response to a signal(s) in the environment. The carboxyl-terminal cytoplasmic region of HK, called transmitter domain, consists of an ATP-binding domain and a so-called H box domain that includes the conserved His residue for self-phosphorylation. Subsequently, the His-bound phosphoryl group of HK is transferred onto a specific Asp residue on the cognate response regulator (RR) for activation. The activated RR activates, in most cases, transcription of a set of genes, which respond to the external signal.

On the basis of *Escherichia coli* genome sequence, a total of 30 HK, each containing the conserved self-phosphorylation domain, and a total of 32 RR, each containing the conserved receiver domain, have been predicted (3). After detailed analysis of the genome sequence, two additional RR candidates, YgeK and YhjB, have been identified, both containing the conserved helix-turn-helix motif of the RR family. Recently, Oshima et al. (4) performed a microarray analysis for a total of 30 TCS mutants of *E. coli* and speculated that, at least for certain combinations, TCSs functionally interact each other to expand the signal transduction network so as to allow some genes to respond to various signals in the environment (4). To examine the specificity of HK-RR interaction in a more direct way, we have purified as many HK and RR proteins as possible, and we tested the self-phosphorylation of HK and the trans-phosphorylation of RR by phosphorylated HK in all possible combinations.

**EXPERIMENTAL PROCEDURES**

**Plasmids**—To construct plasmids for overproduction of the cytoplasmic region of each HK containing the HK catalytic domain and the full-length RR, the corresponding DNA fragments were prepared by PCR using *E. coli* W3110 genome DNA as template and a set of primer pairs (for sequence see supplemental Table I). After digestion of the PCR-amplified fragments with two kinds of the restriction enzyme, each introducing a single cleavage within one of the primer pairs (for sequence see supplemental Table I), the PCR-amplified fragments were inserted into pET21a(+) vector (Novagen) between the same restriction sites as used for the preparation of insert DNAs. All the plasmids thus constructed (supplemental Table II) were confirmed by DNA sequencing.

**Protein Expression**—To achieve high level expression of the target proteins, each plasmid was transformed into three different competent *E. coli* cells, two IPTG-inducible strains, BL21(DE3) and JM109(DE3), and one salt-inducible strain BL21(SI) and selected for one strain.
RESULTS  

Purification of TCS Components—For in vitro analysis of HK-RR interactions, we tried to purify a total of 64 E. coli TCS components (30 HKs plus HK candidates and 34 RRs plus RR candidates including the newly identified YegK and YhjB) in His-tagged forms from overexpressed E. coli. The sensor HK is generally composed of two domains, the amino-terminal membrane-associated domain for monitoring an external signal(s) and the carboxyl-terminal cytoplasmic domain with catalytic function of His phosphorylation. Because the signal molecules for HK activation are not yet identified for some sensors, we decided to purify the cytoplasmic HK domain, which alone retains the activity of autophosphorylation and trans-phosphorylation to the cognate RR in the absence of the effector-binding domain (5–15). The coding sequences for the carboxyl-terminal domain for all 30 sensor HKs or HK candidates with the His6 tag sequence at the carboxyl terminus were PCR-amplified using sets of primer pairs (for sequence see supplemental Table I) and were inserted into pET21a (+) to generate the respective expression plasmids (see supplemental Table I). The expression plasmids for 30 RRs and RR candidates were also constructed in the same procedure by using PCR-amplified coding sequences. Expression plasmids for four RRs, CitB, RbbB, NrcC, and CreB, were constructed from the respective green fluorescent protein fusion-type Archive clones (16) after digestion with NotI for removal of the green fluorescent protein portion. In these cases, the His6 tag sequence is added at the amino terminus of each RR coding sequence.

To achieve the maximum level of protein induction, we first checked the expression level by using three different competent strains, IPTG-inducible BL21(DE3) and JM109(DE3) and salt-inducible BL21(SI), and under various induction conditions with respect to the inducer concentration and the induction time. The host strain and the best conditions for maximum expression differed from protein to protein (data not shown). Three HK proteins, RcsC, YpdA (B2380), and QseC, were not induced in all the hosts used and under all the conditions tested. Except for these three HK proteins, 61 other proteins (27 HKs and 34 RRs) were subjected to large scale expression and purification. Most of the proteins were recovered in soluble fractions and purified to apparent homogeneity by a single step of affinity chromatography. Two HK proteins (AtoS and YehU) and one RR protein (FimZ) were recovered in pellet fractions after centrifugation and thus solubilized in lysis buffer containing 7 M urea. For these proteins, urea was removed after protein purification by dialyzing against storage buffer without urea. As a result, a total of 61 proteins, 27 truncated form HKs and 34 full-length RR, were purified, all of which apparently showed a single band on SDS-PAGE (data not shown).

Self-phosphorylation of HKs—A total of 27 purified HK was subjected to self-phosphorylation in the presence of radioactive ATP. As shown in Fig. 1, 25 species of the purified HK showed the self-phosphorylation activity, but a detectable level of phosphorylation was not observed for two HKs, CitA and YojN. The YojN protein is not an orthodox-type sensor because it lacks the conserved catalytic domain of His kinase, whereas the lack of self-phosphorylation activity for CitA is not yet clear. For all 25 catalytically active HKs, the self-phosphorylation took place rapidly, and the maximum level of phosphorylation was observed within 30 min. The phosphorylation level did not increase after prolonged incubation for up to 2 h (data not shown). The level of phosphorylation, however, decreased after addition of unlabelled ATP (see below), indicating that the self-phosphorylation of HK is a reversible reaction. The dissociation of radioactive phosphorus was also observed even in the absence of ATP. These observations suggest that the HKs used in this study were de-phosphorylated during purification and after prolonged storage in the absence of ATP.

The saturation level of self-phosphorylation at equilibrium ranged from less than 0.01% up to ~5% (the amount of phosphorylated form in the total amount of HK added), assuming that each HK carries a single site of phosphorylation (Fig. 2). The self-phosphorylation level was the highest (4%) for CheA, herein classified as group L1 (level 1). Group L2 with the high activity (higher than 0.03%) includes 14 members (YfhK, TorS, BaeS, RsbT, PhoR, BaaS, YedV, BarA, CreC, PhoQ, UhpB, CusS, KdpD, and DcuS in decreasing order) (Fig. 2). Six L3 group members (NtrB, HydH, CpxA, ArcB, NarX, and EnvZ in decreasing order) exhibited the phosphorylation level of lower than 0.02% and higher than 0.005%. The phosphorylation level was lower than 0.005% for L4 group HK members (NarQ, AtoS, EvgS, and YehU).

The rate of the self-phosphorylation activity, shown as the time (t50%) required for half of the maximum level, can be classified into four groups (Fig. 2). The R1 group showing the fastest rate of phosphorylation (t50%, less than 2 min) includes, in decreasing order, ArcB, BaaS, NtrB, HydH, PhoR, BarA, PhoQ, and TorS (Fig. 2). The R2 group showing the phosphorylation rate (t50%) of 3–5 min includes, in decreasing order, YihK, NarQ, DcuS, RstB, YedV, and CusS. The R3 group showing the rate of 5–10 min includes, in decreasing order, KdpD, EvgS, UhpB, BaeS, NarX, CheA, CpxA, EnvZ, and CreC. Two R4 group members, AtoS and YehU, showed the lowest rate (t50%) of more than 10 min. Both the rate and the level of phosphorylation were very low for AtoC and YehU, supposedly...
because the contents of active HK molecules were low for these preparations.

Among the 25 HK species analyzed, a total of 17 members showed good correlation between the rate ($R$) and the level ($L$) of self-phosphorylation (shaded area in Fig. 2). The activity difference in this group might reflect a difference in the population of active HK molecules in the purified HK preparations.

One of the novel findings in this study was the detection of dephosphorylation activity for HK. Even in the presence of $[^{32}P]$ATP, for instance, the level of phosphorylated NtrB decreased after saturation (see Fig. 1). Five HK members, ArcB (spot A), NtrB (spot Q), NarQ (spot O), NarX (spot P), and EvgS (spot L), showed high rates of phosphorylation but low saturation levels ($R/L$, above the shaded area in Fig. 2). As in the case of NtrB, the HKs of this group may have high activities of dephosphorylation under the reaction conditions employed.

On the other hand, three HK members, CheA (spot F), BarA (spot D), and CreC (spot H) ($R/L$, below the shaded area in Fig. 2), showed high levels of self-phosphorylation even though the rates of phosphorylation were low.

Trans-phosphorylation of RRs by HKs—From both the previously characterized data of E. coli TCS systems and the paired location of HK and RR genes on the E. coli genome, a total of 26 TCS pairs including both CheA-CheB and CheA-CheY have been predicted (3). The genes for five HKs (arcB, barA, narQ, rcsC, and yojN) and six RRs (arcA, fimZ, narP, rcsB, rssB, and uvrY) are not linked to the genes for respective RR partners on the genome. Based on genetic and/or biochemical data, these orphan HKs and RRs have been considered to form the following cognate HK-RR pairs: ArcB-ArcA, BarA-UvrY, and NarQ-NarP (6, 17, 18). The RcsC-YojN and YojN-RcsB systems form a sequential pathway, RcsC-YojN-RcsB, of the His-Asp phospho-relay (19). Up to now, however, no HK partners have been identified for two RRs, FimZ and RssB.

By using a total of 25 functional HKs with self-phosphorylation activity (see Figs. 1 and 2), we performed the trans-phosphorylation assay for all possible combinations. A fixed amount of each HK was first incubated with $[^{32}P]$ATP for self-phosphorylation until saturation (see Fig. 1), and then mixed with an equal molar amount of the respective cognate RR and an excess amount of unlabeled ATP (100-fold molar excess over the radiolabeled ATP). First we examined the HK-RR cognate pairs. Among a total of 26 cognate HK-RR pairs, including both CheA-CheB and CheA-CheY pairs, a significant level of trans-phosphorylation was observed at least for 24 pairs (Fig. 3). EvgS failed to phosphorylate EvgA under the reaction conditions employed, whereas trans-phosphorylation level for AtoS-
Fig. 2. Relationship between the rate and the level of self-phosphorylation of HK. Self-phosphorylation of the purified HKs was carried out under the standard reaction conditions as described in Fig. 1. The intensity of HK-bound $^{32}$P radioactivity was measured with BAS1000 (Fuji Film Co., Japan), and the net amount of phosphorylated HKs was calculated by using the calibration curve, which provides a linear relation between the phosphorylated HK molecule and the HK-bound $^{32}$P radioactivity. The rate of self-phosphorylation is shown as the time (t) required to give half (50%) of the maximum level of phosphorylation. The uppercase letters in each spot corresponds to that in Fig. 1. HKs were classified into four groups (R1 to R4) based on the rate of self-phosphorylation and also into four groups (L1 to L4) on the level of phosphorylation.

AtoC was hard to detect because these two components could not be separated onto SDS-PAGE.

The level of RR phosphorylation was estimated by measuring both the increase in RR-associated $^{32}$P radioactivity and the decrease in HK-associated radioactivity. The results are summarized in Fig. 4. Both the rate and the level of RR phosphorylation were different among the 24 HK-RR pairs. The maximum level of phosphorylated RR was detected less than 5 min after the addition of phosphorylated HK. The decrease of HK-associated radioactivity was fast for 16 HK-RR pairs (group A), including pArcA, pBaeS, pCheA, pCpxA, pCreC, pCusS, pDcuS, pKdpD, pNarX, pNarQ, pNtrB, pTorS, pUhpB, pYehU, and pYfhK (where p represents the phosphorylated form). Concomitantly with the dissociation of $^{32}$P radioactivity from HKs, the respective cognate RRs were phosphorylated. The maximum level of trans-phosphorylation in these cases was observed within <1 min. The lifetime of RR-bound $^{32}$P appears different among RR species. The RR-bound $^{32}$P was stably retained for the A1 group RRs such as pArcA, pCpxR, pCusR, pKdpE, and pNarL but was rapidly released for the A2 group RRs, including pBaeR, pCheB, pCheY, pCreB, pDcuR, pNarP, pNtrC, pTorR, pUhpA, pYehT, and pYfhA. Because the rate of release of RR-bound $^{32}$P was different between the RR species, we concluded that the dephosphorylation rate is an intrinsic property of RR as in the case of HK. Thus, the sum of HK- and RR-bound $^{32}$P showed a time-dependent decrease in most cases, because the dephosphorylation takes place for both phosphorylated HKs and RRs. The rate of trans-phosphorylation was slow for another set of HKs (group B). The cognate RRs, which were phosphorylated by the group B HKs, remained phosphorylated for long periods. After prolonged incubation, the level of phosphorylated RRs increased up to completion for pBaeR, pHydG, pOmpR, pPhoB, and pUvrY (group B1). On the other hand, the trans-phosphorylation stopped in the middle of the reaction for pPhoP, pRstA, and pYedW (group B2), and the phosphorylated form of group B2 HKs apparently stayed unchanged at the time 0 level.

At the time of maximum phosphorylation for each HK-RR pair, the level of phosphorylated RR was also different. High level phosphorylation (higher than 30% of input RR) was observed for CheY and YfhA. The phosphorylation level was from 1% to 10% for TorR, PhoB, CusR, RstA, UvrY, ArcA, BaeR, NtrC, KdpE, UhpA, and PhoP (in decreasing order). The level of phosphorylated RRs was less than 1% for CreB, HydG, YedW, NarL, CheB, OmpR, DcuR, CpxR, YehT, and NarP (in decreasing order).

Cross-talks in Trans-phosphorylation between Non-cognate HK-RR Pairs—Phosphorylation in vivo of RR by non-cognate HK was suggested by genetic and microarray analysis of some HK mutants (4, 17, 20). Here we carried out more systematic and direct search for the cross-talk of trans-phosphorylation of RRs by non-cognate HKs. By using 21 species of the functional HK except for TorS and YehU and 34 species of RR, we examined possible cross-talks in trans-phosphorylation for 692 combinations (21 × 34 − 22 (cognate pairs)). HKs were first self-phosphorylated by incubation with [γ-$^{32}$P]ATP for 30 min and then mixed with equimolar amounts of each non-cognate RR and excess unlabeled ATP. After 30 s of incubation, the mixtures were subjected to SDS-PAGE analysis.

Among a total of 692 non-cognate HK-RR pairs, trans-phosphorylation between non-cognate pairs was identified for a total of 22 combinations, as indicated by the red bars in Fig. 5. Seven species of HK (pBarA, pBaeS, pDcuS, pEnvZ, pRstB, pUhpB, and pYedV) phosphorylated non-cognate RR(s). Among these HKs, pUhpB phosphorylated 9 non-cognate RRs and 1 orphan RR (RstB), pBarA phosphorylated 4 non-cognate RRs, and pDcuS phosphorylated 2 non-cognate RRs, whereas pDcuS, pEnvZ, pRstB, and pYedV phosphorylated each non-cognate RR. The external signals sensed by these HKs must regulate, under certain conditions, the genes, which are under the control of another HK-RR system.

On the other hand, nine species of RR (AtoC, CheY, CusR, HydG, KdpE, NarL, NarP, NtrC, and YfhA) were phosphorylated by non-cognate HKs besides their cognate HKs. Genes under the direct control of these RRs must respond to multiple external signals. CusR was phosphorylated by three non-cognate HKs (pBaeS, pEnvZ, and pRstB), whereas YfhA was phosphorylated by four non-cognate HK (pBaeS, pEnvZ, pRstB,
and $p_{UhpB}$). CheY, NarL, NarP, and YgeK were phosphorylated by two non-cognate HKs. AtoC was phosphorylated by a non-cognate $p_{UhpB}$.

Of the two orphan RRs (FimZ and RssB), of which the pairing HK partners have not been identified, RssB was found to be phosphorylated by three HKs, $p_{ArcB}$, $p_{CheA}$, and $p_{UhpB}$. RssB binds to the stationary phase-specific RNA polymerase $\sigma^S$ (RpoS) and transforms it susceptible to degradation by ClpXP proteases. The results described herein suggest that one of the three HKs is the cognate HK of RssB (phosphorylation by other two HKs must be due to cross-talk in trans-phosphorylation). All these HKs are, however, known to form TCS with the respective cognate RR partner (ArcA for $p_{ArcB}$; CheB and CheY for $p_{CheA}$; and UhpA for $p_{UhpB}$). Phosphorylation of FimZ was, however, not detected with use of any HKs used in this study.

Interference of TCS Signal Transduction by Non-cognate RR—For some specific combinations, the presence of non-cognate RR enhanced dephosphorylation of HK. The enhancement of HK dephosphorylation by non-cognate RR suggests the interference of one TCS signal transduction by another pathway. The combinations of interference are summarized in Fig. 5 (shown by blue lines). Among the RRs tested, five species of RR (CitB, CpxR, PhoB, RssB, and YhjB) enhanced dephosphorylation of more than two species of HK, and four species (CusR, HydG, PhoP, and YfhA) enhanced dephosphorylation of one non-cognate HK. In addition, YhjB, a NarL family orphan RR, stimulated dephosphorylation of 2 HKs, EnvZ and NtrB, even though HK for YhjB phosphorylation was not identified. It is worthwhile to note that RssB, the stability regulator of $\sigma^S$ (RpoS), interacts with and induces dephosphorylation of 6 HKs, including $p_{BaeS}$, $p_{CreC}$, $p_{DcuS}$, $p_{HydH}$, $p_{NarQ}$, $p_{NtrB}$, and $p_{RstB}$.

Dephosphorylation of some HKs was enhanced by multiple species of non-cognate RR. For instance, dephosphorylation of $p_{DcuS}$ was enhanced by four non-cognated RRs (CitB, CpxR,
PhoB, and RssB) and dephosphorylation of $p$EnvZ, $p$NtrB, and $p$RstB was stimulated each by three non-cognate RRs (Fig. 5). Enhancement of HK dephosphorylation by non-cognate RRs may indicate the interference of signal transduction between different TCSs, leading to expand the cross-talk within the signal transduction network.

DISCUSSION

TCS is the signal transduction pathway employed in wide varieties of bacteria. Here we carried out, for the first time, a systematic and comprehensive analysis of the activity and specificity of self-phosphorylation in vitro of HK and trans-phosphorylation in vitro of RR by phosphorylated HK for all purified TCS components from *E. coli*. For both HK self-phosphorylation and RR trans-phosphorylation, the rate and the level of phosphorylation were found to be different among the HK and RR components. The difference in kinetic parameters of HK self-phosphorylation and trans-phosphorylation may, at least in part, correlate with the nature of each HK and RR such as the need for quick response to changes in environment and/or the duration for maintenance of the memory.

In most cases, the HK with a high rate of self-phosphorylation showed a high level of phosphorylation (see Fig. 2). The activity difference might be related, to a certain extent, to the level of functional protein molecules in HK preparations. However, the amount of phosphorylated form in each HK preparation used might be low, if any, because the HK dephosphorylation takes place during purification and storage. Some HKs such as ArcB, NtrB, HydH, and NarQ showed high rates of phosphorlating but low levels of phosphorylation, presumably because these HKs have the high rate of dephosphorylation (Fig. 6) as demonstrated for NtrB (see Fig. 1). In contrast, another group including CheA, BaeS, and CreC showed high levels of self-phosphorylation even though the phosphorylation rate was low (see Fig. 6). The HKs of this group may be able to maintain the phosphorylated state (or the response memory) for long periods. The quick response for the HKs of this group, however, must be archived by another step such as the signal sensing of HK, conformational change of membrane-bound HK, and DNA-binding affinity of RR. The difference in kinetic parameters among the test HKs suggests that the in vitro assay of HK self-phosphorylation reflects, to certain extent, the in vivo situations.

Here we also analyzed the kinetic parameters for trans-phosphorylation of RRs by the cognate HKs. The trans-phosphorylation was experimentally demonstrated in vitro, for the first time, for 9 pairs, CusS-CusR, RstB-RstA, YedV-YedW, BaeS-BaeR, YehU-YehT, Yhk-Yha, HydH-HydG, BasS-BasR, and CreC-CreB (see Fig. 3), supporting the predicted HK-RR pairs.

Both the rate and level of trans-phosphorylation differed between HK-RR pairs. Based on the rate of trans-phosphorylation, HK-RR pairs could be classified into two groups (see Fig. 4). Group A showed high rates of trans-phosphorylation, but the rate was lower for group B pairs. As in the case of HK self-phosphorylation, the rate and the extent of trans-phosphorylation, herein observed, may reflect the nature of signal transduction.
transduction of each TCS. The group A TCSs are able to respond quickly to changes in the environment (see Fig. 6). It is noteworthy that the three HKs (ArcB, NarQ, and NtrB) with a high rate but a low level of self-phosphorylation are all included in this group.

The group A pairs can be further classified into two subgroups with respect to the stability of phosphorylated RR. The phosphorylated RR is stable for group A1 pairs, whereas group A2 pairs are unstable and rapidly dephosphorylated. The group A1 TCSs are capable of maintaining the response memory for long periods. On the other hand, quick dephosphorylation (or inactivation) of the group A2 RRs may lead to rapid loss of the memory, thereby returning to the steady-state of gene expression after transient activation or repression upon exposure to external stresses (see Fig. 6). The instability of phosphorylated RRs could be intrinsic properties but not due to contamination of nonspecific phosphatases, because the addition of HK or RR of group A2 pairs do not accelerate dephosphorylation of RRs from different TCSs (data not shown). The accurate measurement of the rate and the saturation level of trans-phosphorylation are therefore more difficult than those for self-phosphorylation, because HK carries the phosphatase activity of its own phosphate moiety (this work) and the cognate RR-associated phosphate (21). For instance, the level of \( p_{\text{ArcB}} \) is significantly higher than the input \( p_{\text{ArcA}} \), suggesting that loss of radioactive phosphate from \( p_{\text{ArcA}} \) prior to its transfer to ArcB.

Our systematic search for trans-phosphorylation of RRs by non-cognate HKs under the same reaction conditions as used for trans-phosphorylation between the cognate pairs detected the cross-talk in phospho-relay for at least 22 combinations (see Fig. 5). In addition, two of the three combinations, which were newly identified for RssB phosphorylation, might be arisen from the cross-talk. Most interestingly, YgeK, a NarL family RR, is unique, because it lacks the receiver domain, which generally contains the conserved Asp residue that is phosphorylated by HK. Most surprisingly, two HKs, \( p_{\text{BarA}} \) and \( p_{\text{UhpB}} \), phosphorylated YgeK (see Fig. 5), suggesting the presence of novel phospho-relay other than the typical His-3-Asp phospho-
relay. Overall, the total number of cross-talks in E. coli TCS may be 21 (3.0%) out of a total of 692 non-cognate pairs examined. In certain cases, one RR can be phosphorylated by multiple species of HK. Most interestingly, CheY, YfaA, and CusR, which showed high levels of phosphorylation (see “Results”), were phosphorylated by two, four, and three HKs, respectively (see Fig. 5). The target Asp residues on this group of RRs may be 21 (3.0%) out of a total of 692 non-cognate pairs examined. In certain cases, we cannot exclude that the results of cross-talks observed in vitro are artifacts arising from partial denaturation of HKs and/or RRs. However, significant levels of the cross-talk in trans-phosphorylation were observed for a group of RRs (NarP, CpxR, NarL, and HydG), which showed the low level of trans-phosphorylation by the cognate HKs. In these cases, the observed cross-talk may be more specific. For trans-phosphorylation to take place, HKs must carry the conserved segments to function as anchors for their attachment to the conserved segments close to the target Asp residue within RRs (22). The specificity of each cognate HK-RR pair seems to be determined by the variable region(s) close to this HK-RR contact domain. The search for the recognition and interaction sequences for both cognate and non-cognate pairs awaits further analyses.

When one RR is phosphorylated by multiple species of HK, the target genes under the control of this particular RR may respond to various stresses. Likewise, one HK may phosphorylate multiple RRs. In these cases, the intracellular content of RRs, the level of functional forms is controlled by phosphorylation (34), leading to the σ competition (35). The intracellular concentrations of transcription factors vary depending on the culture conditions and/or cell growth phases. In addition, the level of functional forms is controlled by phosphorylation. Both the level and the activity controls of transcription factors lead to governing the global pattern of genome transcription. One attractive hypothesis is that the induced HK dephosphorylation by certain non-cognate RRs is an interference system against competing TCSs.

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Two-component System Signal Transduction Network

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