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Compilation of All Genes Encoding Two-component Phosphotransfer Signal Transducers in the Genome of *Escherichia coli*

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Abstract

Bacteria have devised sophisticated His-Asp phosphorelay signaling systems for eliciting a variety of adaptive responses to their environment, which are generally referred to as the "two-component regulatory system." The widespread occurrence of the His-Asp phosphorelay signaling in both prokaryotes and eukaryotes implies that it is a powerful device for a wide variety of adaptive responses of cells to their environment. The two-component signal transducers contain one or more of three common and characteristic phosphotransfer signaling domains, named the "transmitter, receiver, and histidine-containing phosphotransfer (HPt) domains." The recently determined entire genomic sequence of *Escherichia coli* allowed us to compile systematically a complete list of genes encoding such two-component signal transduction proteins. The results of such an effort, made in this study, revealed that at least 62 open reading frames (ORFs) were identified as putative members of the two-component signal transducers in this single species. Among them, 32 were identified as response regulator and 23 were identified as orthodox sensory kinases. In addition, *E. coli* has five hybrid sensory kinases. The precise location of each ORF was mapped on a physical map of the entire *E. coli* genome. All of these ORFs were then compiled and annotated extensively.

Key words: *Escherichia coli* MG1655; two-component regulatory system; response regulator; sensory kinase

Bacteria have devised sophisticated signaling systems for eliciting a variety of adaptive responses to their environment. These adaptive response systems often involve, at least two components of signal transduction proteins.[1] They are therefore referred to as the "two-component regulatory system."[2] A typical two-component system consists of two types of signal transducers, the "sensory kinase and response regulator."[3] The sensory kinases monitor some environmental parameter, and modulate accordingly the functions of the response regulators. The response regulators mediate changes in gene expression or cell behavior in response to environmental stimuli. Molecular communication between the sensors and their cognate regulators involves phosphotransfer reactions (i.e., His-Asp phosphorelay).[4,5]

Most of these signal transduction proteins contain one of the following two common phosphotransfer signaling domains, the "transmitter and receiver domains" (Fig. 1).[6] The common transmitter domain (ca. 240 amino acids) in the sensory kinases contains several short stretches of amino acids, which are highly conserved in members of the family (Fig. 1A). The transmitter contains an invariant histidine residue, which is autophosphorylated in an ATP-dependent manner. The common receiver (ca. 120 amino acids) in the response regulator serves as a phospho-accepting domain, in which an invariant aspartate residue is located around the center (Fig. 1B). This particular aspartate residue can acquire a phosphoryl group from the phospho-histidine of its cognate transmitter.[7] Besides these transmitters and receivers, another common device has recently been identified, which has also been implicated in phosphotransfer signaling.[8,9] This domain, referred to as the "histidine-containing phosphotransfer (HPt) domain," consists of about 120 amino acids, and contains a short consensus motif in which an invariant histidine residue is located (Fig. 1C). This histidine residue can presumably acquire a phosphoryl group from either of the components, and also transfer it to receivers. The most sophisticated signal transduction proteins of bacteria contain all of the domains in one primary sequence, thereby they can pre-

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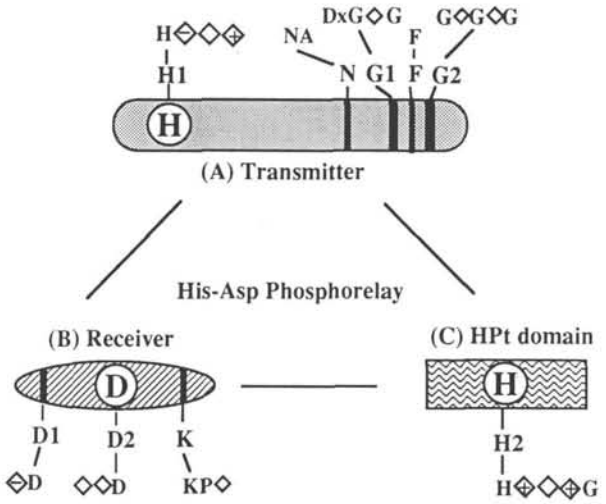

Figure 1. Structural feature of the transmitter, receiver, and HPt domains. Conserved signature motifs are schematically shown for the three kinds of phosphotransfer signaling domains. Letters indicate amino acids present in at least 70% of aligned domains. Diamonds indicate positions at which at least 50% of the amino acids belong to the same chemical family: white, nonpolar (I, L, M, V); plus sign, basic (H, H, R); minus sign, acidic or amidic (D, E, N, Q).[6]

sumably signal in a highly sophisticated manner. This type of signal transducer is referred to as the "hybrid sensory kinase."[10]

To date, members of the two-component systems have been described in a number of different bacterial species.[5] Such a widespread occurrence of the two-component systems implies that it is a powerful device that is used for a wide variety of adaptive responses of bacterial cells to environmental stimuli.[1] Importantly, some have also been discovered in eukaryotes, including plastids, protozoa, fungi, and plants.[8] Furthermore, it has previously been estimated that 40 different sensor-regulator pairs may operate in *E. coli* alone.[5,6,11] The recently determined entire genomic sequence of *E. coli* now allows us to compile systematically a complete list of genes encoding proteins containing either the transmitter, receiver, or HPt domains. According to the results, at least 62 open reading frames (ORFs) were identified as members of the two-component family of signal transducers in *E. coli*.

1. Analyses

To compile a whole list of *E. coli* two-component signal transducers, an extensive computer-aided similarity search was conducted for all *E. coli* ORFs using the current databases (GenBank/EMBL/DDBJ, and Swiss-Prot). The recently released nucleotide sequence database for the entire *E. coli* genome (strain MG1655) was mainly used (accession, gb:AE000111–511), and the extensive database for *E. coli* W3110 was also used as a reference. The BLAST and FASTA search programs were provided by the www severs; NCBI, National Center for Biotechnology Information, USA; GenomeNet, Institute for Chemical Research, Kyoto University, Japan; DDBJ, National Institute of Genetics, Japan.

The amino acid sequences of the well-characterized *E. coli* response regulators, OmpR, NarL, NtrC and CheY, were the first probes used for the similarity search. A large number of *E. coli* ORFs were found to exhibit significant similarity to one of these amino acid sequences. Each amino acid sequence of the probed candidates was then inspected by eye to confirm that each does indeed contain a set of short conserved stretches of amino acids, which are characteristic of the receiver domain (Fig. 1B). A typical receiver of about 120 amino acids should contain at least three signature sequences of amino acids at appropriate distances relative to each other (those designated as D1, D2 and K in Fig. 1B). The D2 sequence contains the presumed phospho-accepting aspartate residue. The results showed that 37 ORFs contained a typical receiver domain.

A further similarity search was conducted also for the transmitter domain, using the amino acid sequences of the *E. coli* EnvZ, NarX, NtrC and CheA sensory kinases as the authentic probes. Assuming that a typical transmitter of about 240 amino acids contains several short stretches of amino acids (those designated as H1, N, G1, F and G2 in Fig. 1A) appropriately spaced relative to each other, a large number of *E. coli* ORFs were confirmed to contain a presumed transmitter domain. The H1 sequence contains the autophosphorylated histidine residue. According to the results, 29 ORFs were predicted to contain a typical transmitter domain.

ORFs containing the HPt domain were the most difficult to find, because the amino acid sequences of the known HPt domains are highly variable (e.g., those in the *E. coli* ArcB and BarA hybrid sensors). However, we previously proposed that an invariant and phosphorylated histidine residue in the HPt-motif should be followed by a short characteristic stretch of amino acids (Fig. 1C).[8] Five *E. coli* ORFs were then predicted to contain an HPt-motif.

Taking these results together, we identified most ORFs, if not all, that contain at least one of the common His-Asp phosphorelay signaling domains, in the whole list of *E. coli* ORFs. We identified 32 putative response regulators containing a receiver, including the well-characterized OmpR and CheY regulators (Fig. 2), 23 putative sensory kinases containing a transmitter, including the EnvZ and CheA sensors (Fig. 4), and five hybrid sensory kinases containing both a transmitter and receiver (and an HPt domain in some) (Fig. 4). In addition, an ORF seems to have an HPt domain, preceded by

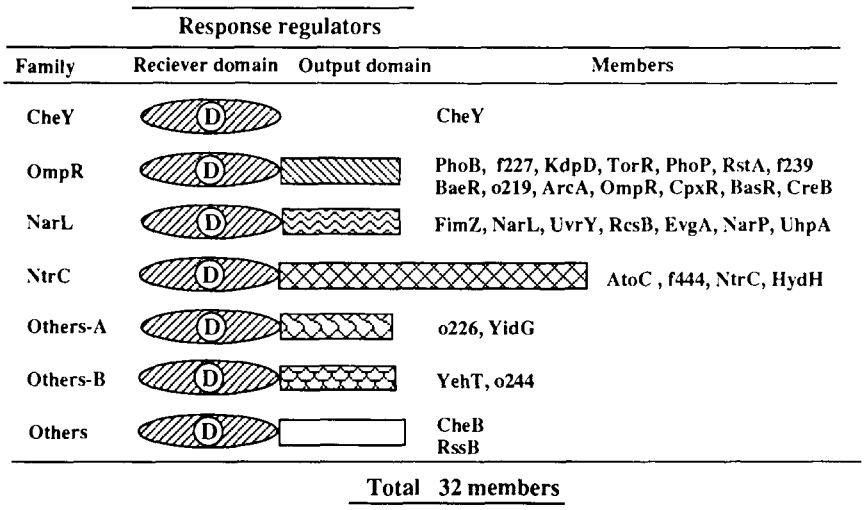

Figure 2. List of response regulators of *E. coli*. The regions corresponding to the receiver and output domains, respectively, are shown schematically.

an atypical transmitter sequence lacking the phosphorylated histidine residue (Fig. 4, YojN). The precise location of each ORF was mapped on a physical map of the entire *E. coli* MG1655 genome (Fig. 5). All of these ORFs were then compiled and annotated extensively (Table 1).

2. Response Regulators

Thirty-two members were identified as response regulators containing a receiver (Fig. 2). All of these response regulators, except for CheY, have a similar structural design; a common receiver domain is followed by an output domain speific to each. An exception is CheY, which consists of only a receiver domain. A cross-examination of the sequences of the respective output domains revealed that they can be classified into distinct subgroups. Fourteen ORFs exhibited extensive similarity to OmpR, not only in the receiver domains but also in their output domains (data not shown). As judged by the same criteria, seven others appear to belong to members of the NarL-subfamily (see the alignment in Fig. 3A). Similarly, four other ORFs are members of the NtrC family. It should be noted that the OmpR, NarL and NtrC response regulators are known to function as specific DNA-binding transcriptional regulators. It can thus be assumed that these 25 members are DNA-binding transcriptional regulators, as has been demonstrated for some, including PhoB, KdpD, ArcA, NarP. These response regulators are implicated in gene regulation through phosphotransfer signaling in response to some environmental factor, and each is postulated to regulate (activate or repress) a specific subset of genes in *E. coli*.

Four newly identified members (o226, YidG, YehT and o244) are similar to neither OmpR, NarL nor NtrC, in their amino acid sequences of the presumed output domains. Among them, however, o226 and YidG show extensive similarity to each other throughout the entire sequences, including their presumed output domains (data not shown). The same is true for the pair of YehT and o244. The remaining two, CheB and RssB, are unique in the sense that each has a unique output domain; the former has esterase activity involved in the chemotactic signal transduction[1] whereas the latter is somehow implicated in the proteolytic degradation of sigma-factor (σ^S) through the function of the ClpXP protease in *E. coli*.[12]

3. Sensory Kinases

Twenty-three ORFs were identified as orthodox sensory kinases containing a transmitter, whose structural designs are similar to each other, in which a typical transmitter domain is preceded by a presumed N-terminal signal-input domain (Fig. 4, and also see Table 1). Note that each input domain of these sensory kinases is unique with regard to the amino acid sequence and length, thereby each may serve as a specific signal transducer, as has been demonstrated for a number of these members including EnvZ, NarX and NtrB. The CheA chemotactic sensor alone has a unique structural design, as has been well documented previously.[6]

The amino acid sequences of the transmitters in YehU and o565 are quite divergent from those of authentic transmitters. That is, the BLAST and FASTA programs gave a very low similarity score relative to other members, and in fact, these ORFs are not annotated as a putative sensory kinase in the original databases. How-

Figure 3. Alignments of amino acid sequences showing similarities of some signal transducer proteins. A, an alignment of the output domains of the NarL family of response regulators. B, an alignment of the HPt domains found in *E. coli*. The presumed phospho-histidine site is indicated by an asterisk. C, a certain portion of the YojN amino acid sequence was aligned with that of the RcsC transmitter domain. Note that the G1 and F motifs appear to be present in YojN as well.

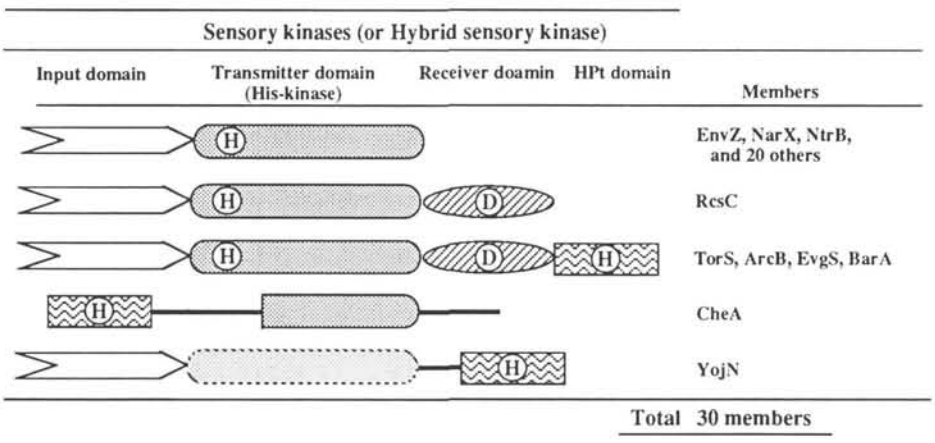

Figure 4. List of sensory kinases and hybrid sensory kinases of *E. coli*. The regions corresponding to the transmitter, HPt and input domains, respectively, are shown schematically. Details are given in the text.

ever, a close inspection revealed that they contain a set of the signature motifs (very divergent though), including the phosphorylated histidine site. In fact, they exhibit a significant similarity to a subset of sensory kinases, which were reported previously for other species (Table 1). Furthermore, on the *E. coli* genome, each of these ORFs reside next to YehT and o244, respectively, which have been identified as a typical response regulators, as mentioned above (Figs. 2 and 5).

4. Hybrid Sensory Kinases

E. coli has five hybrid sensory kinases, RcsC, TorS, ArcB, EvgS and BarA, as reported previously.[8] RcsC

Table 1. Compiled list of ORFs involved in phosphotransfer signaling in *E. coli*.

Sen	gulator[a]	gb:Accession[b] (nt)		Combination[c] (dir)	Remarks	Relevant adaptive Systems [putative] and [Accession]
Om	nily					
	PhoB	AE000146	(411739)	RS(+)		Phosphate regulation
	227	AE000162	(592447)	RS(−)		[CopS/R, *Pseudomonas syringae*, copper response] [sp:COPS_PSESM]
	/KdpE	AE000173	(719952)	SR(−)		Potassium transport
	TorR	AE000201	(1050390)	RxS(−)	Hybrid	Trimethylamin metabolism
	'PhoP	AE000213	(1184913)	RS(−)		Stress situations
	RstA	AE000256	(1676082)	RS(+)		?
	239	AE000288	(2030342)	RS(−)		[CzcS/C, *Alcaligenes eutropha*, heavy metal homeostasis] [gp:AECZDRS_9]
	BaeR	AE000297	(2144629)	SR(+)		[AFQ2/1, Streptomyces coelicolor, second metabolism] [sp:AFQ2_STRCO]
	219	AE000384	(3158965)	RS(+)		[YgiY/X, *Haemophilis influenzae*, unkown] [sp:YGIY_HAEIN]
		AE000400	(3343888)	S(−)	Hybrid	Respiratory control
	ArcA	AE000510	(4632550)	R(−)		Respiratory control
	OmpR	AE000416	(3530158)	RS(−)		Osmotic regulation
	'CpxR	AE000466	(4100038)	RS(−)		Maltiple systems
	BasR	AE000483	(4322514)	RS(−)		Virulence
	CreB	AE000510	(4632550)	RS(+)		Catabolite repression
Nar	ily					
	Z	AE000159	(555801)	R(−)	Orphan	[−/FimZ, *Salmonella typhimurium*, fimbrial expression] [sp:FIMZ_SALTY]
	NarL	AE000220	(1269078)	SR(−)		Nitrate regulation
	Y	AE000284	(1985995)	R(−)	Orphan	?
	RcsB	AE000311/310	(2314690)	RxS(+/−)	Hybrid	Capsule synthesis
	EvgA	AE000325	(2478347)	RS(+)	Hybrid	[BvgS/A, *Bordetella pertussis*, virulence] [sp:BVGS_BORPE]
		AE000333	(2573751)	S(+)		Nitrate regulation
	NarP	AE000309	(2288295)	R(+)		Nitrate regulation
	'UhpA	AE000444	(3837592)	RS(−)		Hexose phosphate uptake
Ntr	ily					
	AtoC	AE000311	(2314690)	SR(+)		Acetoacetate metabolism
	144	AE000342/341	(2687352)	RxS(+)		?
	NtrC	AE000462	(4047390)	RS(−)		Nitrogen regulation
	'HydG	AE000473	(4186952)	SR(+)		Labile hydrogenase activity
Che	ily					
	CheB/CheY	AE000282	(1964286)	SxxxxRR(−)		Chemotaxis
Oth						
	226	AE000167	(651283)	SR(+)		[−/CriR, *Shigella flexneri, ipa* genes expression] [gp:SFU29054_1], and see below
	YjdG	AE000485	(4342802)	SR(−)		[CitA/B, *Klebsiella pneumoniae*, citrate metabolism] [prf:2204370B]
Oth						
	YehT	AE000301	(2202210)	SR(−)		[LytS/T, *Bacillus subtilis*, autolysin response] [gp:BSZ75208_9]
	244	AE000326	(2491273)	SR(+)		[−/MrkE, *Klebsiella pneumoniae*, fimbrial expression] [sp:MRKE_KLEPN]
Oth						
	B	AE000222	(1289390)	R(+)	Orphan	Sigma-S degradation
		AE000310	(2301690)	(S)(+)	HPt	?
	–	AE000362	(2907549)	S(+)	Hybrid Orphan	Pilus adherence

[a] Cognate pa sensor/regulator are classified and listed. [b] The GenBank accession numbers for the sequeunces of the set of 400 *E. coli* chromosomal segments are indicated, in of which the ponding gene(s) is located. Note that *nt* indicates the position of the first nucleotide of each registered sequence with respect to the total number of nucleotides c *E. coli* chro e (4638858 -bp). [c] The combination of each pair of sensor/regulator on the chromosome is indicated (SR, 5′-sensor/3′-regulator; RS, 5′-regulator/3′-sensor). Note *dir* indicates lative direction of transcription on the chromosome (+, direct; −, complementary).

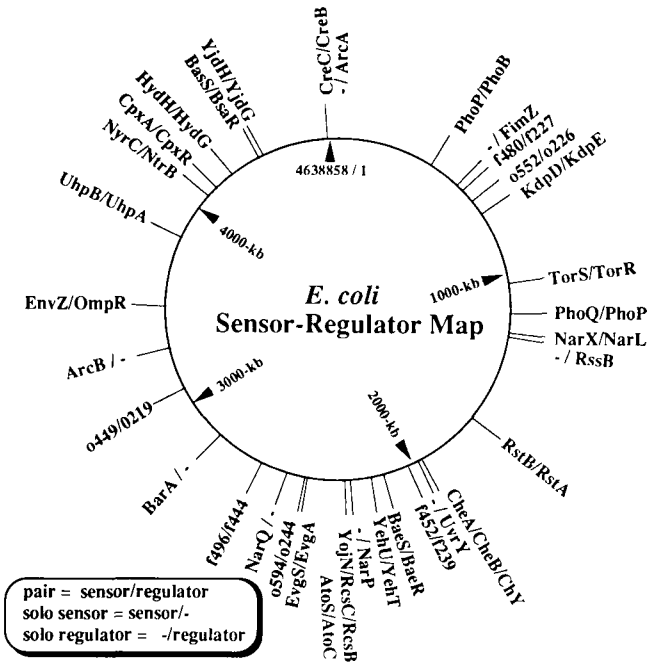

Figure 5. Mapping of the positions of ORFs, each of which was predicted to encode one of the signal transducer proteins. This is based on the physical map of strain MG1655 (see Table 1).

contains both the transmitter and receiver domain, while the other four contain an HPt as well (Fig. 4). The corresponding amino acid sequences of these HPt-motifs are aligned, together with the autophosphorylated histidine site of CheA (Fig. 3B). It is clear that they are significantly similar to each other, and contain the invariant phosphorylated histidine site. Although no new hybrid sensory kinase emerged in this study, an inspection of such an HPt domain revealed an intriguing ORF, YojN (Table 1). In the C-terminal region of the YojN sequence, a typical HPt-motif was found (Figs. 3B and 4). Interestingly, an amino acid sequence of about 200, upstream of the C-terminal HPt domain, is somewhat similar to the transmitter domain (see the alignment in Fig. 3C). Some signature motif (e.g., G1 and F) appears to be present in YojN, yet a presumed phospho-histidine site could not be assigned in YojN. This may function as an as yet unknown type of signal transmitter in the His-Asp phosphorelay. It may also be worth mentioning that the gene encoding YojN is located very closely to the *rcsC* and *rscB* genes, which respectively encode the RcsC sensory kinase and the RcsB response regulator (Figs. 5 and 6).

5. Cognate Sensor/Regulator Pairs

The chromosomal positions of each coding sequence specifying one of these identified signal transducers are scattered evenly over the genome of MG1655 (Fig. 5 and Table 1, see the column of accession). In most instances, however, a certain cognate pair of sensor/regulator is located next to each other, and most likely, in the same transcriptional unit (or operon) (Fig. 5, and Table 1, see the column of sensor/regulator). Interestingly, the order of these pairs of genes (5′-sensor/3′-regulator or 5′-regulator/3′-sensor) and the transcriptional direction relative to the chromosome (direct or complementary) appears to be random (Table 1, see the column of combination). In this respect, two particular pairs (i.e., ArcB/ArcA and NarQ/NarP) are exceptional in the sense that each corresponding partner resides at a different location of the chromosome, although each pair is known to function together in a certain signaling pathway (Fig. 5). In any case, it is tempting to speculate that each cognate sensor/regulator pair is most likely functions in a specific signaling pathway, as has been demonstrated for a number of cases in *E. coli* (Table 1, see the column of relevant adaptive systems).

An *E. coli* chromosomal region (at approximately 2315 kb) contains a cluster of genes each encoding a sensor or a regulator (Fig. 6). The *yojN* (HPt domain), *rcsB* (regulator), *rcsC* (hybrid sensor), *atoS* (sensor) and *atoC* genes form a contiguous cluster. The *rcsB/rcsC* pair is involved in capsule synthesis,[13] whereas the *atoS/atoC* pair is implicated in acetoacetate metabolism.[14] *yojN* is a unique example of *E. coli* signal transducers, as mentioned above. These facts may or may not be meaningful. It is also noteworthy that, in this particular region of *E. coli* strain W3110, the *rcsC* gene is split by an IS2-insertion (*ca.* 1.3 kb), as indicated in Fig. 6.

Out of 29 sensors or hybrid sensors, only BarA is an orphan in terms of its functional partner. Out of 32 regulators, only 3 are lonely (i.e., FimZ, UvrV and RssB). The physiological function of BarA was recently suggested to be involved in pilus adherence during host infection.[15] One of these three regulators may or may not be the partner of BarA. In any case, it is of interest to look for their partners, *vis a vis*.

The above mentioned situation regarding the chromosomal organization of the signal transducer genes is in direct contrast to the case of *Synechocystis* PCC 6803, for which we recently compiled all genes encoding signal transducers.[16] *Synechocystis* PCC 6803 has 38 response regulators and 42 sensory kinases, including a variety of hybrid sensory kinases. Of these 70 genes, 48 (60%) reside apart from their presumed partners on the chromosome. In any case, a more deep inspection with special reference to the chromosomal organization of these putative redundant genes is of interest from the evolutional point of view, although this is outside the scope of the present study.

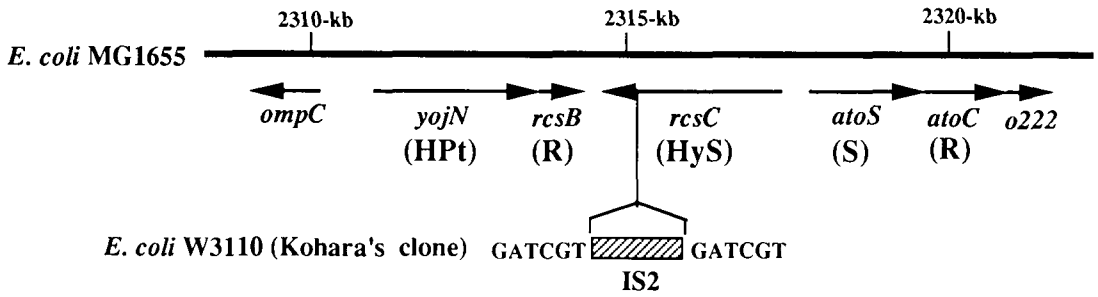

Figure 6. A certain region in the *E. coli* chromosome contains a cluster of genes encoding a sensor or a regulator (S, sensory gene; R, regulator gene; HyS, hybrid sensor gene; HPt, protein containing an HPt domain). Note that strain W3110 appears to have an insertion of the IS2 segments.

6. Relevant adaptive systems

In Table 1, each identified ORF (or each cognate pair of ORFs) were classified and annotated. Most of these annotated facets are mentioned above. Here the physiological relevance of these *E. coli* signal transducers is much concerned (see the column of relevant adaptive systems). For the 16 sensor/regulator pairs, their physiological functions in certain adaptive systems were experimentally documented previously, to some extent.[6] The function of the triplet, CheA/CheB/CheY, is involved in the chemotactic behavior, and is best characterized in *E. coli*.[1] The function of the BarA sensor and the RssB regulator have also been characterized,[12,15] although their respective partners are not known, as mentioned above. The remaining ten sensor/regulator pairs and two solo regulators remain to be characterized.

To help in addressing these relevant issues more deeply, a further computer-aided analysis was conducted. As mentioned above, each signal input domain of the unknown sensory kinases has its own context with regard to the amino acid sequence. In fact, the amino acid sequences of the input domains of these *E. coli* sensory kinases do not significantly resemble each other, and thereby presumably are able to serve as a specific signal transducer. Thus, an extensive similarity search was carried out using only the specific N-terminal sequences of those particular unknown sensors as the probes. This was carried out in the hope of finding a presumed homologue within other bacterial species, for which a possible function has already been suggested (if they have a similar input domain, they might respond to a similar signal). In certain cases, this type of approach successfully revealed a putative homologue of each *E. coli* unknown sensor (Table 1, see the parentheses of the column of adaptive systems). For example, the f480 sensor has an N-terminal signal input domain, the amino acid sequence of which is significantly similar to that of the CopS sensor of *Pseudomonas syringae*, which was previously reported to be implicated in a response to external copper in this bacterium.[17] The EvgS sensor has a signal input domain, the amino acid sequence of which is significantly similar to that of the BvgS sensor of *Bordetella pertussi*, which is known to be related to virulence.[18] A similar approach, conducted for the certain unknown regulators (FimZ, o226 and o244), revealed also a similar response regulator in other bacterial spices. These allowed us to put an annotation for some *E. coli* unknown sensor/regulator or regulator, as indicated in Table 1. However, it should be emphasized that these annotations are solely putative (and in some case, the significance of the observed similarity was less clear). Nevertheless, these may give us hints to clarify the physiological function of the yet unknown sensors and regulators of *E. coli*.

In this study, we compiled most of the members, if not all, that belong to the widespread two-component signal transducers, based on the currently available entire nucleotide sequence of the *E. coli* genome. These compiled data should provide us with, at least, preliminary hints to systematically characterize a whole network of the His-Asp phosphorelay signaling in *E. coli*. This approach may be useful as a post-sequencing step.

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