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Function, Diversity, and Evolution of Signal Transduction in Prokaryotes

Meeting Review

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Major areas covered at the Bacterial Locomotion and Signal Transduction (BLAST) meeting included the clustering of chemoreceptors and its significance to signal amplification, organelle biogenesis, motility, developmental responses mediated by "chemotaxis" operons, and advances in two-component signaling mechanisms.

Prokaryotes use their small size and metabolic diversity to dominate every conceivable niche on earth. A large part of this success comes from evolution of elaborate sensory systems to monitor and respond to their environment, to direct motility, and to program development. Research over the last decade has led to a fairly sophisticated understanding of many of these signaling mechanisms in the model organisms *Escherichia coli* and *Salmonella typhimurium*. The influence of the genomics era on these studies was clearly evident at the seventh biennial Bacterial Locomotion and Signal Transduction (BLAST) meeting held in January 2003 in halcyon surroundings, in the mellow, sun-soaked, mountainous city of Cuernavaca, Mexico (http://www.uic.edu/orgs/blast/).

Subcellular Organization of Supramolecular Signaling Complexes in Chemotaxis

Bacterial chemotaxis is arguably the first complete behavioral system in biology to be understood at the molecular level, yet the potential for complexity in chemoreceptor signaling is just being realized (Falke, 2002). *E. coli* possesses five different transmembrane receptors (MCPs), which sense specific signals, generally in the periplasm. They are present in differing amounts in the cell, the more abundant ones in several thousand copies. Six different cytoplasmic components all interact with the cytoplasmic domains of the MCPs to form a supramolecular signaling complex (Figure 1). The signaling state of the MCPs regulates the autophosphorylation activity of the histidine kinase CheA, whose association with the MCPs is stabilized by the coupling protein CheW. In its active state, CheA phosphorylates two response regulators—CheY and CheB. Phosphorylated CheY (CheY∼P) diffuses to the flagellar motor, where it regulates swimming activity by interacting with the switch protein FliM. CheZ accelerates dephosphorylation of CheY∼P. Adaptation to the sensory stimulus is mediated by methylation and demethylation of the cytoplasmic domains of MCPs, catalyzed by the methyltransferase CheR and the phosphorylated form of the methylesterase CheB, respectively. CheA-CheY and CheA-CheB are kinase response regulator members of a large family of two-component signaling proteins.

In many species of bacteria, chemoreceptors are predominantly localized at one cell pole, forming an enormous cluster (Figure 1; Gestwicki et al., 2000; Maddock and Shapiro, 1993). Although the basic chemoreceptor subunit is a symmetric dimer of identical subunits, the crystal structure of the soluble cytoplasmic domain reveals a trimeric assembly of three symmetric dimers (Kim et al., 1999). Signal propagation through higher-order complexes of these trimers of dimers could account for the observed (but poorly understood) signal amplification in chemotaxis, where very small changes in receptor occupancy produce a substantial chemotactic response (Duke et al., 2001; Segall et al., 1986; Sourjik and Berg, 2002). Measurements of CheA kinase activity versus attractant concentration produce Hill coefficients ranging from 1 to 3, consistent with this view of interacting receptors (Bornhorst and Falke, 2001; Sourjik and Berg, 2002). Experimental work, primarily with *E. coli*, provides growing support for the notion that the receptor arrays represent functional signaling units (Ames et al., 2002).

Role of Receptor Clustering

Interactions between neighboring MCP molecules may significantly enhance receptor-stimulated kinase activity by a mechanism not yet understood (Shimizu et al., 2000). In membranes prepared from cells coexpressing Tar and Tsr receptors, a synergistic increase in CheA kinase activity, measured by accumulation of CheY∼P, was seen in vitro (Run-zhi Lai, Texas A&M University). This result suggests that mixed receptor populations enhance activation of CheA kinase and may explain how low-abundance receptors signal in conjunction with Tar or Tsr. By supporting a high rate of CheY∼P production, mixed-receptor patches may also promote turnover of CheY∼P to allow a faster response to attractants.

Disulfide crosslinking of cysteine mutants of the Tar receptor in vivo revealed a molecular weight consistent with a trimer of dimers (Ikuro Kawagishi, Nagoya University, Japan). Quantification of the levels of each of the Che proteins and the chemoreceptors, in different strains and different culture conditions, indicates that

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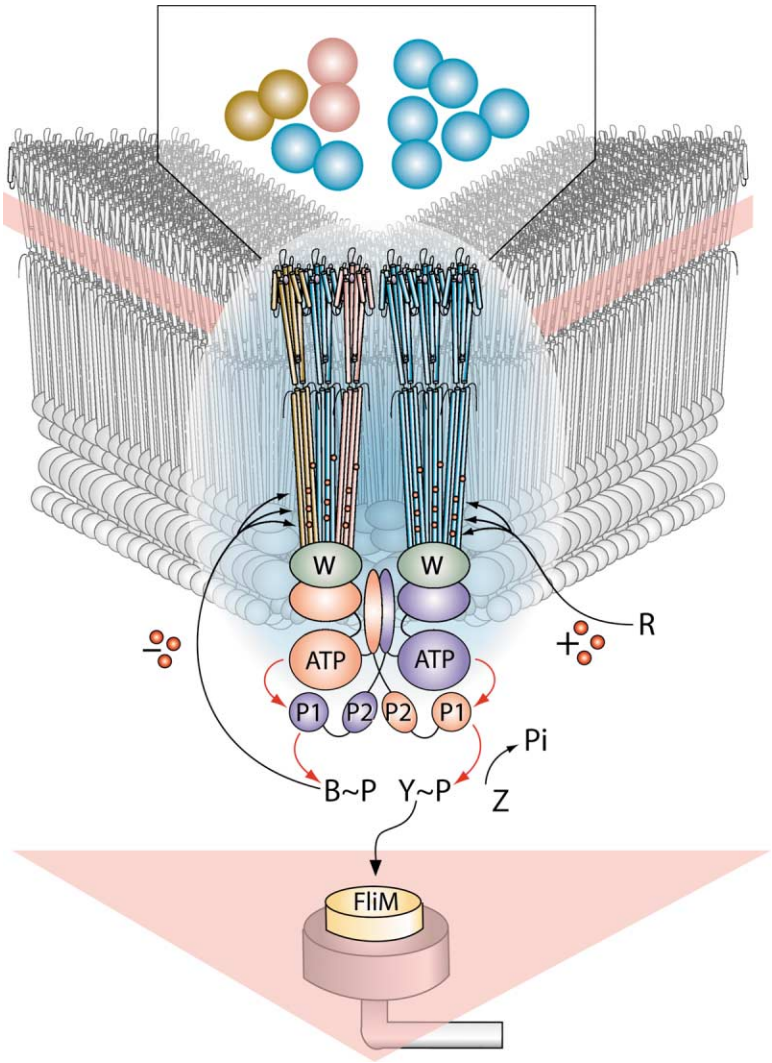

Figure 1. A Pie-Shaped Slice through an *E. coli* Cell to Reveal Polar Clusters of Transmembrane Chemoreceptors

Two trimers of receptor dimers are highlighted. They can exist in pure or mixed arrangements (see inset). Higher-order configurations of the trimer of dimers are thought to result from binding to CheW adaptors, linked, in turn, to CheA kinase in a giant sensory scaffold. CheA plays a central role not only in signal transduction, but also in targeting soluble components to the clusters, and possibly in the spread of conformational signals through the network. A His residue on the P1 domain of the dimeric CheA mediates phosphotransfer to CheY and CheB, which dock on CheA via the P2 domain. The direction of phosphotransfer is indicated by red arrows. CheR and CheB add and remove CH_3 groups (red circles). The pink stripes and triangle represent the inner membrane. Adapted from Stock and Levit (2000) and Falke (2002).

there is substantial variation in the levels of these proteins, but the ratio of the components with respect to one another is relatively constant (Gerald Hazelbauer, University of Missouri). Images of higher-order complexes of the cytoplasmic domain of Tsr complexed with CheW and CheA indicate that the receptors may interact end to end as well as side to side, implying that receptor signals may be integrated via both axial and lateral contacts (Peter Wolanin, Princeton University; Weis et al., 2003).

A simulation of chemotaxis in vivo was presented by Thomas Shimizu (Keio University, Japan). Previous models based on kinetic parameters can reproduce many features of the attractant response but fail to obtain the experimentally observed gain. The new simulation incorporated a spatial interaction between variously methylated receptors. The outcome was significantly closer to recent in vivo results, suggesting that this mechanism could account for the observed signal amplification, but discrepancies remain in the precise form of the dose-response relation between attractant concentration and kinase activity.

Targeting Soluble Signaling Components to Receptor Clusters

The kinase activity of CheA is maximally expressed in ternary complexes with MCPs and CheW (Figure 1). With GFP fusions, it has been shown that all the other proteins in the chemotaxis pathway colocalize with the MCP clusters (Sourjik and Berg, 2000). Many of these interactions occur through the multiple domains of CheA (Figure 2). A CheB-GFP fusion is targeted via the N-terminal domain of CheB to the P2 domain of CheA (Satomi Banno, Nagoya University, Japan). Similarly, CheZ-GFP is targeted to the clusters by binding to the truncated P1 domain of the short form of CheA (CheAs; Brian Cantwell, Texas A&M University). Immunoprecipitation experiments were consistent with this conclusion (Christopher O'Connor, University of Illinois, Chicago).

Chemical modification of cysteine-substituted CheA proteins reveals that the CheW binding surface of the P5 domain is occluded by the ATP binding domain in the CheA crystal structure, implying that the various domains of CheA undergo substantial conformational

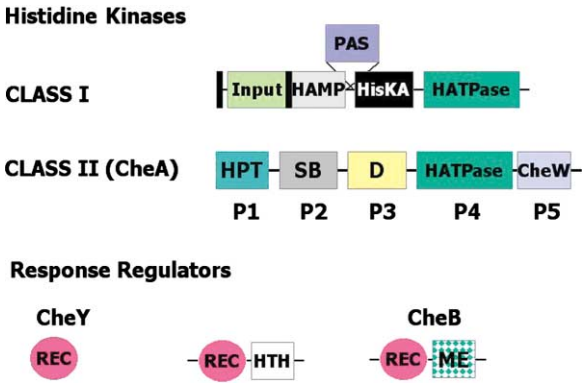

Figure 2. Modular Design of Histidine Kinases and Response Regulators

The black rectangles indicate transmembrane domains. Most two-domain response regulators have a C-terminal DNA binding domain, but a few have enzymatic activity (e.g., the methylesterase of CheB). The domains and their functions are as follows (domain, function): input, ligand binding; HAMP, linker (named for presence in histidine kinases, adenylyl cyclases, MCPs, and phosphatases); PAS, redox sensing, cofactor binding (named for presence in Per, Arnt, and Sim); HisKA, dimerization (contains the phosphorylated histidine); HATPase, ATP binding; HPT, histidine phosphotransfer (contains the phosphorylated histidine); SB, substrate binding (CheY/CheB); D, dimerization; CheW, regulatory coupling; REC, response regulator receiver (contains the phosphorylated aspartate); HTH, helix-turn-helix DNA binding; ME, methylesterase.

shifts in solution, for example, during assembly of receptor signaling complexes (John Parkinson, University of Utah).

Comparative genomic analysis indicates that bacteria use a wide variety of domain organizations of these proteins (Igor Zhulin, Georgia Institute of Technology). One of the unexpected findings was that many CheA homologs lack the P2 domain and instead have a response regulator phosphorylation domain at their C termini. Further bioinformatics and experimental analyses of such domain organization will provide important clues to the diversity of molecular interactions in chemotaxis.

Cytoplasmic Clustering

Rhodobacter sphaeroides has three Che operons with multiple homologs of the *E. coli* sensory proteins, most of which are required for chemotaxis (Porter et al., 2002). The chemoreceptors are located in two clusters: polar clusters, as in *E. coli*, and cytoplasmic clusters that localize to a distinct region (Wadhams et al., 2002). Remarkably, the cytoplasmic clusters have the appearance of organelles that are actively segregated upon cell division. George Wadhams (University of Oxford, UK) presented evidence that each cluster is functionally distinct and that a specific subset of chemotaxis proteins is localized to each site. The polar complex was shown to be composed of several proteins encoded by CheOperon2 (CheW2, CheW3, CheA2, and CheR2), whereas the cytoplasmic cluster contained many products of CheOperon3 (CheW4, CheA3, and CheA4). The cytoplasmic CheA3 and CheA4 histidine kinases form a functional heterodimer (Steven Porter, University of Oxford, UK). Given that the various chemosensory proteins may be differentially expressed under different growth conditions, the distinct clusters may respond to distinct external and internal environmental and metabolic cues.

Organelle Biogenesis and Motility

Surface Motility

Bacteria move over surfaces by a variety of mechanisms. While flagella and type IV pili (TFP) mediate swarming and retractile motility, respectively, mechanism(s) underlying the gliding movement of many bacteria are not understood (McBride, 2001). Social gliding, or S motility, in *Myxococcus xanthus* is mediated by TFP and regulated by at least three *che* clusters. The *frz* cluster controls cell reversal frequency, during which TFP alternate extension and retraction from opposite cell poles, while the *dif* cluster is responsible for production of extracellular polysaccharide "fibrils" essential for S motility (Shimkets, 1999; Yang et al., 2000). Yinuo Li (University of California, Los Angeles) reported that fibrils provide attachment sites for TFP and trigger pili retraction. Polysaccharides such as chitin (made of N-acetylglucosamine) mimic this bioactivity, suggesting that similar residues in the fibrils serve as anchors for attachment of the tips of extended pili prior to retraction. An attachment organelle is also important for gliding motility in mycoplasmas, parasitic bacteria that lack the peptidoglycan layer, and do not have flagella, pili, or a chemosensory system (Miyata et al., 2002). Several mycoplasma species have a distinct cell polarity and glide in the direction of the tapered end. Makoto Miyata (Osaka City University, Japan) reported that *M. mobile*, which glides continuously on glass, exerts a force up to 27 pN. Freeze-fracture EM has identified several 50 nm-long spikes that stick out from the cell membrane around the neck of this organism and grab the glass surface. With a combination of nonbinding and nongliding mutants, as well as monoclonal antibodies that inhibit gliding, two large protein targets (349 and 521 kDa) with distinct functions were localized to the neck. Gli349 is required for glass binding, while Gli521 is required for movement. A mechanical cycle of gliding was proposed, where Gli349 is involved in attachment, while Gli521 is responsible for the stroke or force generation that achieves gliding speeds of 2–4.5 μm/s.

Organelle Biogenesis

Biogenesis of locomotive organelles, such as pili and flagella, is a challenging task. These structures are assembled from the inside out, so proteins have to be transported over large distances from the site of synthesis, particularly during assembly of the external filament, which is several times the body length of the bacterium. Construction and function of locomotive organelles represent a substantial commitment of energy resources. Since motile species are generally endowed with the capacity to direct their movement in beneficial directions, environmental cues regulate transcription in order to turn off the synthesis of all these genes in optimal environments where the need for migration ceases. A remarkable mechanism exists for regulating flagellar biogenesis in *E. coli* and *S. typhimurium*, where sequential assembly of the flagellum is brought about by sensing various stages of completion of the organelle itself

(Aldridge and Hughes, 2002). The partially completed structures serve as checkpoints in morphogenesis, signaling sequential expression of the appropriate flagellar operons arranged in a three-tiered transcriptional hierarchy. This regulatory strategy minimizes waste in case of unforeseen blocks in assembly. A flagellum-specific type III secretion system (TTSS), which is homologous to the secretion apparatus for virulence proteins in many pathogenic bacteria, allows the ordered exit of components through the hollow core. The Hughes lab has characterized an anti-sigma factor that couples completion of hook assembly to expression of sigma 28-dependent genes needed late in assembly; it is actively secreted via the completed hook basal body, freeing sigma 28 to transcribe late-assembly genes (Hughes et al., 1993). Three factors influence secretion through the TTSS—the N-terminal sequence of the exported protein, chaperones that bind to their substrates and stabilize them for export, and upstream elements of mRNA implicated in coupling translation to assembly. Philip Aldridge (University of Washington, Seattle) described several specific substrate-chaperone pairs. The chaperones have dual roles and also regulate transcription or translation of specific substrates in other pairs needed at different stages of assembly. This not only provides another sensing mechanism to determine the stage of flagellar assembly, but also leads to a complex regulatory feedback, where expression of the three transcriptional tiers oscillates. Heather Bonifeld's (University of Washington, Seattle) results suggest that translation of the flagellar filament gene may be targeted to the base of the flagellum itself, and Shin-Ichi Aizawa (CREST, JST, Japan) presented EM evidence for the presence of flagellin at these sites.

A Cautionary Tale of Two Locations

In *Caulobacter crescentus*, cell differentiation and asymmetric cell division produce two cell types with distinct developmental programs: a stalked cell and a motile and chemotactic swarmer cell. To replicate and divide, motile swarmer cells have to differentiate into stalked cells. During this process the chemotaxis machinery, the flagellum and pili, are lost and are replaced by a stalk and adhesive holdfast structure at the same pole. During the divison cycle, the cell assembles a new flagellum and pili at the pole opposite the stalk (Poindexter, 1964). Several two-component systems control polar organelle development and coordinate it with the cell cycle. Some of these, including the kinases PleC, DivJ, and CckA, localize to specific cell poles and undergo dynamic rearrangements as a function of the cell cycle (Jenal and Stephens, 2002). Stephen Sciochetti (Princeton University) has identified the polar targeting signals of the stalked pole-specific kinase DivJ (Sciochetti et al., 2002) and, at this meeting, described the targeting signal of the swarmer cell pole-specific kinase PleC. A DivJ-PleC fusion protein containing the targeting sequences of DivJ and the catalytic domain of PleC kinase localized to the stalked pole yet was able to restore motility and stalk formation in a *pleC* mutant. These results suggest that the "correct" polar address of the PleC kinase may not be critical for all its regulatory functions. Interestingly, both PleC and DivJ kinases signal to the unorthodox response regulator PleD, which contains a widespread, but poorly characterized, GGDEF, or DUF1, domain. GGDEF proteins are implicated in the metabolism of the cofactor ci-diGMP (Tal et al., 1998). Evidence presented by Urs Jenal (University of Basel, Switzerland) suggested that ci-diGMP might serve as a possible second messenger for regulating motility and development in *C. cresentus*.

Chemotaxis Operons as Mediators of Developmental Responses to External Stimuli

Role in Development

The first indication that chemotaxis genes may control other behaviors came from studies on swarming in *E. coli* and *S. typhimurium*, where these genes were shown to be required for swarmer cell development and surface motility (Burkart et al., 1998). Chemotaxis, per se, is not important for swarming in these organisms. Qingfeng Wang (University of Texas, Austin) presented evidence that, in the absence of other chemotaxis genes, a constitutively active form of CheY is sufficient to confer swarming ability. Microarray analysis showed that approximately 100 genes were differentially regulated in a *cheY* mutant, some of which belonged to flagellar operons. However, CheY$\sim$P is unlikely to be directly involved in regulating gene expression. Suppressors of a nonswarming *cheY* mutant mapped to the flagellar motor switch protein FliM, leading to possible models of how CheY-FliM interaction affects swarming.

Control of motility is critical to the developmental cycle of *M. xanthus* (Shimkets, 1999). Nine Che operons are now discerned in this organism, which displays two kinds of motilities—adventurous (A) and social (S) motility. While Che1 (Frz system) controls both A and S motilities, Che4 was reported to exclusively modulate S motility (Hera Vlamakis, University of California, Berkeley). The Che2, or Dif, operon has been known to control fibril biogenesis essential for bringing cells together during S motility (Yang et al., 2000). John Kirby (Georgia Institute of Technology) showed that Che3 plays a role in developmental gene expression. Mutations in several Che3 genes show premature and inappropriate expression of developmentally regulated genes but do not affect motility. Interestingly, there is no CheY homolog in this operon, but a divergently transcribed gene, *crdA*, is homologous to NtrC-like response regulators. CheA3 and CrdA apparently interact, and CrdA mutants are delayed in development. Periplasmic stress was implicated as a possible signal here and in parallel pathways regulating development (Heidi Kaplan, University of Texas Medical School, Houston). Thus, Che operons control gene expression for both motility and development in *M. xanthus*.

A developmental role for a chemotaxis operon in *Rhodospirillum centenum* was presented by James Berleman (Indiana University). There are three Che operons in this organism, all controlling some aspect of motility. Che3 plays an additional role in cyst formation, a starvation response. Mutation of several Che3 genes, including *cheY*, results in hypercyst formation in rich medium but does not affect motility of vegetative cells.

Role in Biofilm Formation

Pseudomonas aeruginosa is an opportunistic human pathogen with five Che operons and 26 MCP genes (Stover et al., 2000). One set of genes is involved in flagellar chemotaxis, and another set is important for

pilus-mediated motility. Carrie Harwood (University of Iowa) reported that a third set of *che* genes (called cluster II) has a minor involvement in chemotaxis and is instead required for biofilm formation. Che II genes are expressed in the stationary phase of cell growth. Microarray studies showed that expression levels of a common set of 116 *P. aeruginosa* genes are altered in *cheA*2, *cheB*2, and *cheY*2 mutants. This result suggests that a signal transduction complex comprised of cluster II Che proteins may modulate gene expression, rather than cell motility. Genes for anaerobic respiration were among those whose expression was most dramatically affected. The interior of a biofilm is likely to be starved for oxygen, and mutants that are unable to carry out anaerobic denitrification may form biofilms with altered structures. A challenging task will be the identification of upstream signals for the multitudinous MCPs, as well as mechanisms by which response regulators that lack DNA binding domains alter developmental programs.

Genomic Perspective

CheA is a member of the class II histidine kinase family (HKII) of proteins (see Figure 2; Bilwes et al., 1999). Igor Zhulin (Georgia Institute of Technology) reported that, of 111 bacterial genomes surveyed, over half had at least one HKII. Interestingly, all genomes possessing at least one CheA homolog also have at least one MCP, implying that the two may function together. All bacteria containing at least one CheA are motile, but none of the nonmotile bacteria have a CheA, suggesting that the ancestral chemotaxis system is involved in motility. Over half of the motile bacteria possess more than one HKII. Typically, these bacteria have more than one complete chemotaxis system. It is apparent that chemotaxis genes have also acquired a function typical of HKI kinases, which control a wider variety of cellular processes (Robinson et al., 2000).

Finally, the recently sequenced genome of *Wolinella succinogenes*, an obligate enterointestinal symbiont, revealed that it has the highest percentage (5.5% of all genes) of signal transduction genes of any bacterium analyzed so far (Stephan Schuster, Max-Plank Institute, Tubingen, Germany). Functional analyses of the pathways involved should illuminate mechanisms used by microorganisms not only for their survival and growth, but also for transmission into larger host populations.

Advances in Two-Component Reaction Mechanism

Two-component regulatory systems represent the major paradigm for signal transduction in prokaryotes and lower eukaryotes. The simplest forms contain a sensor kinase (HKI) that is phosphorylated on a conserved histidine residue (see Figure 2). The phosphoryl group is transferred to a conserved aspartic acid residue on the receiver domain of the second component, the response regulator. Most commonly, response regulators are composed of two domains, with the effector, or output, domain binding to DNA.

Interdomain Interfaces

In all species, protein modification is a common mechanism for altering activity. Many response regulators bind to DNA with higher affinity when phosphorylated, although some use phosphorylation to drive dimerization or to relieve inhibition of the C-terminal effector domain. Major questions in the field are, what are the structural consequences of phosphorylation in the receiver domain, and how is the information transmitted to the effector domain? A significant advance was the determination of the full-length structure of the response regulator DrrB from *Thermotoga maritima* (Victoria Robinson, University of Medicine and Dentistry, New Jersey). It is apparent that the interdomain interface between the receiver domain and the effector domain, across which information must be transmitted, is as varied as the number of structures that have been solved to date (four thus far). Different surfaces are utilized in each interdomain interface. Thus, there is no conserved formula for activation that results from phosphorylation, and the repertoire is astonishingly varied. Clearly, patterns will only become apparent after many more structures have been determined. These results also provide a cautionary note about the use of chimeric proteins that may disrupt the signaling interface.

Further advances came from the structural determination of the individual N-terminal receiver domain of PhoP from *Bacillus subtilis* (Catherine Birck, CNRS, France) and the central ATPase domain of NtrC from *Aquifex aeolicus* (David Wemmer, University of California, Berkeley). The interprotein surfaces of PhoP are asymmetrical, leaving a surface on each monomer of the dimer to drive oligomerization, creating a novel mode of association between receiver domains. A salt bridge between D60 on one surface and R113 on the other is critical for this interaction and for function (Chen et al., 2003). NtrC is a response regulator that interacts with the σ^{54} form of RNA polymerase. Phosphorylation results in rearrangement of the dimer to form a different interaction surface and formation of a ring. While seven monomers were found in the ring in the crystals of the ATPase domain alone, there was considerable discussion of the possibility that, in vivo, with the other domains present, there may only be six (David Wemmer, University of California, Berkeley).

Identification of Targets of Essential Genes

Only one of the 35 two-component systems in *B. subtilis* (a gram-positive, soil-dwelling bacterium) is essential. The YycF/G system is required for bacterial survival and cell division and is specific to "low G+C content" bacteria, including many major pathogens, such as *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Listeria monocytogenes*. The genes that are regulated by this system are largely unknown. For the identification of the targets of YycF, a novel approach used a chimera in which the phosphorylation (receiver) domain of PhoP was linked to the DNA binding domain of YycF. With the known signal for PhoP (phosphate starvation conditions), an autolysin gene regulated by YycF was identified. DNase I footprinting with purified native protein identified a 6 bp consensus repeat, and a search of the genome revealed additional potential members of the regulon, including a number of genes involved in cell wall synthesis and cell division (Tarek Msadek, Pasteur Institute, France). Extending the genomic analysis to *S. aureus* suggests that a number of potential virulence factors may be regulated by this essential two-component regulatory system. It therefore may represent a promising potential therapeutic target.

Physical Sequestration of the Regulator

In *C. crescentus*, an established mechanism for controlling signaling is to sequester, or compartmentalize, the components. A variation on this theme was reported, in which the *E. coli* ROK transcriptional regulator (of *r*epressors, *O*RFs, and *k*inases) Mlc was prevented from interacting with the transcriptional machinery by binding to the transporter PtsG (Winfried Boos, University of Konstanz, Germany). Under glucose-replete conditions, PtsG is phosphorylated and Mlc represses transcription of genes and operons encoding sugar-metabolizing enzymes and uptake systems. Under low-glucose conditions, PtsG donates its phosphoryl group to glucose, enabling Mlc to bind and inactivating its repressor functions. Thus, the location of the regulator is intimately tied to the active state of the transporter.

Signaling and Energy Coupling

Many sensor kinases and most MCPs contain a conserved structural element called the HAMP linker, located between the transmembrane region and the histidine kinase domain (see Figure 2). It was suggested that the HAMP linker is a negative regulator of output domain activity. Deletion mutations in each of the amphipathic α helices in the HAMP linker (AS-1 and AS-2) had radically different phenotypes. Deletions in AS-1 diminished HAMP function, whereas AS-2 deletions resulted in a reversed-response phenotype, suggesting that the helices play unique roles in signaling (J. Alex Appleman, University of California, Davis).

Another regulatory element, the PAS domain, is found in some histidine kinases and often binds a cofactor, such as heme, FAD, or cinnaminic acid, depending on whether signaling is in response to oxygen, redox potential, or light, respectively. The Aer sensor kinase contains both HAMP and PAS domains. Second site suppressor analysis of Aer suggests that the HAMP domain contacts the PAS domain and stabilizes FAD binding. Interactions between HAMP domains across the dimer interface (HAMP/HAMP′) as well as HAMP/PAS interactions are crucial for FAD binding and normal Aer signaling (Kylie Watts, Loma Linda University).

In *Rhodobacter capsulatus*, RegB senses oxygen by monitoring redox and signaling to the response regulator RegA (Lee Swem, Indiana University). A reactive cysteine residue near the active site affects autophosphorylation of RegB by ATP. The reduced form of RegB is the active form, and metal binding promotes formation of the oxidized form. The reactivity of the cysteine is probably enhanced by two basic flanking residues. The kinase is sensitive to many divalent metal ions, suggestive of a structural role of the metal in promoting formation of the inactive form.

Links to the Eukaryotic World

The reliance on two-component signal transduction distinguishes the prokaryotes from higher eukaryotes. However, the strategies utilized by bacteria to coordinate environmental signals are so diverse that fruitful comparisons to eukaryotic cells can be found.

Perhaps the most compelling strategy is the utilization of receptor arrays for signal amplification. The details elucidated in bacteria have implications for the assembly of large membrane protein complexes. The notion that clustering of receptors can amplify signals downstream of those receptors resonates within the eukaryotic community. This type of amplification is likely to occur in T lymphocytes during assembly of the "immune synapse." It may recapitulate some of the signal amplification that must occur within the postsynaptic densities within neuronal cells that contribute to synaptic plasticity. This type of clustering may occur in all cells with specialized membrane microdomains, for example, during maturation of lipid rafts into functional signaling units. This process is likely to involve a similar consolidation of membrane-associated signaling proteins. Furthermore, the mechanisms of transactivation triggered by receptor clustering may provide insights into activation of eukaryotic receptors, perhaps best exemplified by transactivation of heterodimers of the receptor tyrosine kinases.

The appreciation of methylation in eukaryotic signaling is in its infancy and represents one area that might well follow leads developed in bacterial systems. The concept of checkpoints during organelle synthesis might have implications in the rapid regulation of neurite outgrowth, although the need for rapid reversibility of organelle synthesis in bacteria may suggest that this type of regulation is unique to prokaryotes. The mechanisms that bacteria have developed for integrating modular assembly and traffic of organelles with the signal transduction machinery will certainly find parallels in the eukaryotic field of microtubular trafficking, where cargo proteins interact directly with signal transduction proteins. The studies in bacteria suggest that these interactions are necessary to provide the assembly machinery with a continuous reading of the environmental demands for the particular assembly. It will be very interesting to determine whether similar feedback mechanisms regulate cargo assembly and traffic in eukaryotes and whether parallels between the two worlds will also be found in mechanisms for targeting of mRNA translation to sites of organellar assembly.

Lastly, phosphorylation strategies utilized by many two-component systems directly affect DNA binding. This situation mirrors the mechanism of eukaryotic *s*ignal *t*ransducers and *a*ctivators of *t*ranscription (STAT) proteins. As additional prokaryotic strategies are unraveled, they will certainly serve to stimulate the imagination of scientists working in signal transduction in all organisms.

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