pH Sensing in *Bacillus subtilis*: a New Path to a Common Goal

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ABSTRACT pH is one of the most fundamental properties of the environments in which microorganisms live. It is, therefore, not surprising that bacteria have evolved mechanisms to sense and respond to pH. One aspect of this response for motile bacteria is to migrate to areas of optimal pH. The paper by P. Tohidifar, M. J. Plutz, G. W. Ordal, and C. V. Rao (J Bacteriol 202:e00491-19, 2020, <https://doi.org/10.1128/JB.00491-19>) describes how *Bacillus subtilis* uses bidirectional chemotaxis mediated by four closely related dCACHE_1 chemoreceptors to migrate to regions of neutral pH.

KEYWORDS *Bacillus subtilis*, chemotaxis, dCACHE_1 chemoreceptors, pH sensing

Temperature and pH are properties of all of the vast diversity of habitable environments in nature. It is therefore hardly surprising that the abilities to monitor and respond to these ubiquitous physical and chemical parameters are major elements of the sensory repertoire of all free-living bacteria. In the laboratory, we can carefully control these factors to optimize growth and survival. Out in the cold—or hot—cruel world, bacteria have to fend for themselves. An important part of the behavior of motile bacteria is the ability to navigate within temperature and pH gradients to find their optimal conditions.

The majority of signal transduction systems are composed of proteins, and the structure and function of proteins can be exquisitely sensitive to temperature and pH. It is therefore not surprising that many different receptors and many different molecular mechanisms can be utilized as thermometers and pH meters. In this issue, Tohidifar et al. (1) report that the common and widespread soil bacterium *Bacillus subtilis* recruits four different methyl-accepting chemoreceptors (McpA, McpB, TlpA, and TlpB) to navigate to locations of neutral pH. *B. subtilis* combines the countervailing activities of McpA and TlpA, which respond to decreasing pH as an attractant signal, and McpB and TlpB, which respond to increasing pH as an attractant signal, in order to migrate to the happy medium of pH 7. I presume that it is a fortuitous coincidence that the acid receptors both end in A and the base receptors end in B.

All four receptors are encoded by genes in a single chromosomal cluster (2), and it is likely most or all of them have functions other than monitoring pH. Of the two dominant pH-sensing receptors, McpA is a repellent receptor for indole, and TlpB is an attractant receptor for amino acids. The first interesting conclusion is that at least four of the ten chemoreceptors of *B. subtilis* are involved in pH sensing, when it seems that one or two might suffice. It is also noteworthy that the genes of the four characterized pH sensors are all located adjacent to one another, as though there is a pH-sensing locus. The four receptors share a great deal of sequence similarity, and it seems likely that they arose by two successive gene duplications. It is also likely that they share a mechanism of pH sensing, as worked out in this study for McpA and TlpB.

The extracellular domains of all four pH-sensing chemoreceptors belong to the dCACHE_1 family (3), the most widely distributed and one of the best-characterized superfamilies of chemoreceptor ligand-binding domains. The extracellular domain of each monomer in a homodimeric receptor contains two CACHE modules, one membrane distal and the other membrane proximal. The membrane-distal module has been

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shown to be the binding site for ligands such as amino acids. Until the present study, the role of the membrane-proximal module remained mysterious. It was thought, perhaps, to function solely in propagation of conformational changes in the membrane-distal module caused by ligand binding to transmembrane signaling. Now we know that the membrane-proximal module also can serve directly in detecting extracellular pH.

One of the primary virtues of this paper is that it describes a well-conceived series of experiments that lead logically from one to another. The initial assays showed that wild-type cells migrate to capillaries containing buffer at pH 7 from ponds of either pH 6 or pH 8. They also showed that this is a bona fide chemotaxis response, as it depends on the three known chemotaxis adaptation systems, most notably on the primary one involving CheRB-mediated receptor methylation/demethylation.

The next step was to identify the pH sensors. Strains that expressed only a single one of the ten chemoreceptors were tested. McpA mediated attractant responses to decreasing pH and was therefore assigned an acid receptor. McpB and TlpB both mediated attractant responses to increasing pH and thus were considered to be base receptors. The remaining receptors did not mediate pH chemotaxis, but when the weakly expressed TlpA receptor was expressed from the much stronger *mcpA* promoter, it also served as an acid receptor. It remains possible that the remaining six receptors could mediate pH taxis if they were overexpressed, but this was not tested.

The single-receptor expression study was complemented with an examination of the responses of cells deleted for individual receptor genes. As expected, cells deleted for *mcpA* showed a drastic decrease in the ability to be attracted to low pH, cells deleted for *tlpA* showed a modest decrease in the sensing of low pH, and cells deleted for both genes were unable to respond to low pH. Conversely, cells deleted for *mcpB* or *tlpB* showed decreased attractant response to high pH, and cells deleted for both genes were totally defective in base sensing. Thus, two independent methods reinforce one another in characterizing the pH sensing activities of the four receptors in question.

The next step was to construct chimeras with different combinations of amino acid sequences from the receptors and to express them as the sole chemoreceptor. In the initial set, the N-terminal portion was contributed by TlpB and the C-terminal portion by McpA. Every receptor in which the entire N-terminal extracellular domain was from TlpB functioned as a base receptor. Interestingly, a fusion at position 260 functioned both as an acid and as a base receptor, indicating that the region of extracellular McpA between residues 260 and 284 is involved in the acid response of McpA. A chimera with the fusion joint at residue 180 served only as an acid receptor, indicating that the region between residues 180 and 284 of McpA contained everything needed for acid sensing and that the region of TlpB between those residues contains the determinants for base sensing. A number of "sandwich" chimeras were also constructed to narrow down the regions required for sensing acid and base, and the final conclusion was that regions between residues 197 and 222 and 260 and 284 contained the critical residues for pH responses.

Examination of the predicted tertiary structure of McpA and TlpB showed that residues 199 and 200 were in close proximity to residues 273 to 274 in both folded proteins. These residue pairs were Thr-Gln/His-Glu in McpA and Lys-Glu/Gln-Asp in TlpB. The authors hypothesized that hydrogen bonding between these residue pairs could be affected by the protonation state of the ionizable residues. The detailed data are given in the paper, but a quick summary of the results from residue swapping at these positions showed that TlpB could be converted into an acid sensor and McpA into a base receptor simply by exchanging the residues at these positions. Although McpB and TlpA were not examined in this detailed manner, base-sensing McpB has Lys-Glu/Lys-Asp as residues 200 to 201 and 274 to 275, and acid-sensing TlpA has Lys-Glu/His-Asp at these positions. Thus, base-sensing McpB has a similar pair of residue dyads at these positions as TlpB. It is of particular interest that TlpA, which can act both as an acid and base sensor, has the same residue dyad at positions 199 to 200 as TlpB and a very similar dyad at positions 273 to 274 as McpA. Figure 6 in the paper presents a

simple model for both acid and base sensing by protonation/deprotonation-dependent hydrogen bonding between these residue pairs. The distribution of residues in the respective proteins drives attractant signaling to decreased pH in McpA and to increased pH in TlpB. Changes in hydrogen bonding may drive conformational changes in the membrane-proximal dCACHE_1 domain that could be propagated across the membrane and through the cytoplasmic HAMP domain to induce phosphorylation of CheA, phosphotransfer to CheY, and activation of smooth swimming and an attractant response by interaction with FliM at the flagellar motor.

A natural question arises as to how broadly distributed this mechanism for pH sensing may be. A bioinformatic analysis presented in Fig. 7 indicates that it may be very widespread, being highly conserved within different *Bacillus* species but also across 50 genera, 12 families, 5 orders, 4 classes, and 3 phyla, including mostly *Firmicutes* but also a few *Proteobacteria* and *Actinobacteria*. It is very satisfying that such a straightforward and general mechanism has been elucidated, which likely holds for most, if not all, of the species identified by bioinformatics and no doubt many other uncharacterized species as well.

Life, of course, cannot really be as simple as that. The enteric bacteria *Escherichia coli* and *Salmonella enterica* also show biphasic chemotaxis to pH that leads them to near-neutral (pH 7.5) environments. They use the opposite responses mediated by the high-abundance amino acid receptors Tar and Tsr (4). These receptors belong to the four-helix bundle group of extracellular chemoreceptor domains, which are very different in structure and, presumably, signaling mechanism from the dCACHE_1 receptors. *Helicobacter pylori* uses the pH-dependent binding of urea to its PAS (sCACHE_2) extracellular sensing domain (5).

The authors note that there are probably other strategies that have evolved for pH sensing, which seems likely for two reasons. First, pH is a universally important environmental signal. Second, the structure of proteins can be affected in a large number of ways by the protonation and deprotonation of the Asp, Glu, Lys, Arg, and His residues, keeping in mind that the pK_a of these residues is very dependent on the sequence context in which they appear (6). I briefly consider titling this commentary "One way to skin a cat: bidirectional pH sensing in *Bacillus subtilis*." Surely, the possibilities for pH sensing are more numerous than the ways to skin a cat. It is good to have a detailed description of one of them.

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