

MicroReview

Influence of pH on bacterial gene expression

Eric R. Olson

*Department of Biotechnology, Parke–Davis
Pharmaceutical Research Division, Warner–Lambert
Company, 2800 Plymouth Road, Ann Arbor, Michigan
48105, USA.*

Summary

Bacteria respond to changes in internal and external pH by adjusting the activity and synthesis of proteins associated with many different processes, including proton translocation, amino acid degradation, adaptation to acidic or basic conditions and virulence. While, for many of these examples, the physiological and biological consequence of the pH-induced response is clear, the mechanism by which the transcription/translation machinery is signalled is not. These examples are discussed along with several others in which the function of the gene or protein remains a mystery.

Overview

A hallmark of microbial life is the ability to sense, respond to and, when necessary, alter the environment. Investigation of these activities has been a major driving force leading to our understanding of regulatory circuits within the cell. Historically, many of these circuits were unravelled by first identifying changes in the expression of a particular gene which in turn led to the identification of the cell surface elements which initially receive the signals. Progress in dissecting the cell's response to pH has taken the opposite course. Because of the intricate relationship between proton gradients and the energized state of the cell, our understanding of what happens at the membrane in response to changes in pH outpaces our knowledge of any resulting changes in gene expression. The history of industrial microbial fermentations for the production of metabolites is filled with examples in which pH has been shown to play a crucial role. No doubt if the molecular mechanisms were known many of these effects could be traced back to pH-dependent changes in gene expression.

Several good reviews covering bacterial pH homeostasis and the physiological and bioenergetic consequences of pH changes have been published (Booth, 1985; Foster, 1992; Guffanti and Krulwich, 1984; Ingledew, 1990; Kroll, 1990; Krulwich, 1986; Krulwich and Guffanti, 1983; 1986; 1989; Krulwich *et al.*, 1990; 1988; Padan and Schuldiner, 1986; 1987; Padan *et al.*, 1981; Slonczewski, 1992). Many concepts laid out in these reviews form the basis for much of what is covered here. What distinguishes this communication, however, is an emphasis on studies in which pH has been implicated in altering the expression of a gene. The paradigms described hopefully serve to illustrate some of the themes that have emerged from studies published in the last few years, as well as framing the major issues yet to be resolved.

Table 1 contains a list of genes whose expression has been shown to be influenced by pH. The most glaring omission is lack of representation from acidophiles and alkalophiles, organisms which present unique opportunities for understanding pH-mediated responses. The elegant physiological and biophysical analyses that have taken place over the past decade with these bacteria provide a strong theoretical foundation for genetic approaches designed to identify the genes and regulatory mechanisms involved. Based on known effects of pH, however, expression of several classes of genes would be predicted to be altered as a consequence of pH in these organisms (Ingledew, 1990; Kroll, 1990; Krulwich and Guffanti, 1989). For example, Ingledew points out that because of the solubility of Fe(III) as a function of pH (10^{23} difference between pH 2.0 and pH 10.0) acidophiles must have mechanisms for tolerating high concentrations of iron while alkalophiles must have effective methods of obtaining it (Ingledew, 1990). Of particular interest will be investigations with facultative organisms since they must be able to adapt to both neutral and extremes in environmental pH.

For most of the examples listed in Table 1, only the ends of the pathways have been identified, a change in pH representing the initial signal and a change in the synthesis rate of a protein the final step. For this reason, detailed discussion of mechanistic aspects at the level of gene expression is not possible. Therefore, the current challenge is to elucidate the molecular mechanisms involved at the ends of the pathways, identify the components that

Gene/Protein	Process/Function	Organism	Reference
<i>aceF</i>	Dihydrolipoamide acetyltransferase	<i>E. coli</i>	Heyde and Portalier (1990); Hickey and Hirshfield (1990)
<i>adi</i>	Arginine decarboxylase	<i>E. coli</i>	Auger <i>et al.</i> (1989); Tabor and Tabor (1985)
<i>alx</i>	?, induced by alkaline pH	<i>E. coli</i>	Bingham <i>et al.</i> (1990)
<i>cadA, cadB</i>	Lysine decarboxylase, lysine/cadaverine antiporter	<i>E. coli</i>	Auger and Bennett (1989); Auger <i>et al.</i> (1989); Meng and Bennett (1992a,b); Tabor and Tabor (1985); Watson <i>et al.</i> (1992)
<i>cydAB</i>	Cytochrome <i>d</i> oxidase	<i>E. coli</i>	Cotter <i>et al.</i> (1990)
<i>cyoABCDE</i>	Cytochrome <i>o</i> oxidase	<i>E. coli</i>	Cotter <i>et al.</i> (1990)
<i>groEL, dnaK, htpG, htpM, grpE</i>	Stress proteins	<i>E. coli, S. typhimurium</i>	Abshire (1991); Foster (1991); Heyde and Portalier (1990); Taglicht <i>et al.</i> (1987)
<i>inaA</i>	?, induced by membrane-permeable weak acids	<i>E. coli</i>	Slonczewski <i>et al.</i> (1987); White <i>et al.</i> (1992)
<i>lamB</i>	Maltose transport	<i>E. coli</i>	Heyde and Portalier (1987)
<i>lysU</i>	Lysyl-tRNA synthetase	<i>E. coli</i>	Hickey and Hirshfield (1990); Hirshfield <i>et al.</i> (1984); L��v��que <i>et al.</i> (1991)
<i>lysP (cadR)</i>	Lysine permease	<i>E. coli</i>	Steffes <i>et al.</i> (1992)
<i>malE</i>	Maltose-binding protein	<i>E. coli</i>	Heyde <i>et al.</i> (1991)
<i>nhaA</i>	Na ⁺ /H ⁺ antiporter	<i>E. coli</i>	Karpel <i>et al.</i> (1991); Pinner <i>et al.</i> (1992); Rahav-Manor <i>et al.</i> (1992)
<i>ompF, ompC</i>	Porins	<i>E. coli, S. typhimurium</i>	Foster and Hall (1990); Heyde <i>et al.</i> (1991); Heyde <i>et al.</i> (1988); Heyde and Portalier (1987)
<i>polA</i>	DNA polymerase I	<i>E. coli</i>	Hickey and Hirshfield (1990)
<i>speF</i>	Ornithine decarboxylase	<i>E. coli</i>	Kashiwagi <i>et al.</i> (1991); Tabor and Tabor (1985)
Proteins on 2-D gels observed by various low pH treatments	?, some involved in adaptation	<i>E. coli, S. typhimurium</i>	Abshire (1991); Foster (1991); Foster and Hall (1990); Hassani <i>et al.</i> (1991); Heyde and Portalier (1990); Hickey and Hirshfield (1990)
Random	?	<i>E. coli</i>	Heyde <i>et al.</i> (1991)
<i>phm::phoA</i> gene fusions			
SOS genes	DNA repair	<i>E. coli</i>	Schuldiner <i>et al.</i> (1986)
<i>aniG</i>	?, co-induced by external acid and mannose	<i>S. typhimurium</i>	Aliabadi <i>et al.</i> (1988); Foster and Aliabadi (1989)
<i>pag</i> genes	Virulence factors, macrophage survival	<i>S. typhimurium</i>	S. I. Miller, personal communication; S. I. Miller (1991); Abshire (1991)
<i>vir</i> genes	Bacterial-host interactions	<i>A. tumefaciens</i>	Chen and Winans (1991); Mantis and Winans (1992a); Mantis and Winans (1992b); Stachel <i>et al.</i> (1986); Turk <i>et al.</i> (1991); Winans (1990); Winans (1992)
Acid-induced proteins observed on gels	?	<i>A. tumefaciens</i>	Mantis and Winans (1992a)
<i>nodA, nodF</i>	Nodulation	<i>R. leguminosarum biovar trifolii</i>	Richardson <i>et al.</i> (1988)
<i>hrp</i> genes	Pathogenic response on plants	<i>P. syringae</i> pv. <i>phaseolicola</i> , <i>E. amylovora</i>	Rahme <i>et al.</i> (1992); Wei <i>et al.</i> (1992)
ToxR regulon	Virulence factors	<i>V. cholerae</i>	DiRita (1992); DiRita <i>et al.</i> (1991); Mekalanos (1992); V. L. Miller <i>et al.</i> (1987); Parsot and Mekalanos (1991)
<i>menCD</i>	Menaquinone synthesis	<i>B. subtilis</i>	Hill <i>et al.</i> (1990)
<i>agr</i> and Agr-regulated genes	Regulation of exoprotein synthesis	<i>S. aureus</i>	Regassa and Betley (1992)
Arginine deiminase	Arginine catabolism	Streptococci	Burne <i>et al.</i> (1991); Cunin <i>et al.</i> (1986)
H ⁺ -ATPase	ATP synthesis/hydrolysis	<i>E. faecalis</i>	Kobayashi <i>et al.</i> (1986)
Metabolic switch from acidogenic to solventogenic fermentation	Production of butanol	<i>C. acetobutylicum</i>	Rogers (1986)

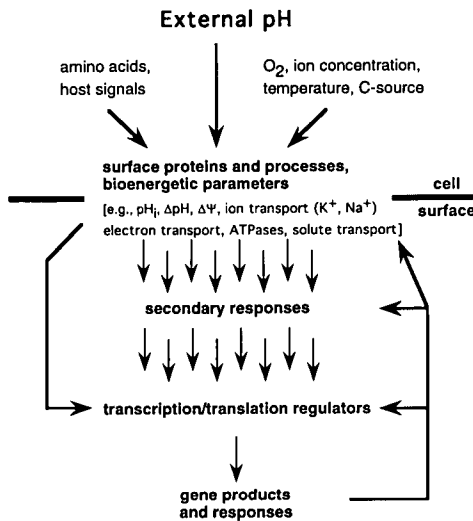


Fig. 1. Information flow between elements involved in pH-mediated gene expression.

lie in between, understand how they bisect other pathways, and integrate these concepts into a fuller understanding of how the organism behaves in nature.

pH as a stimulus

A major difficulty in dissecting the mechanisms responsible for pH-mediated changes in gene expression is that diverse but interdependent sets of signals are generated when the external or internal pH changes (Fig. 1; for a discussion of these parameters see Futai and Tsuchiya, 1987; Padan and Schuldiner, 1987; Kaback, 1986). Depending on the organism and growth condition, changes in external pH (pH_o) can bring about subsequent alterations in several primary physiological parameters, including internal pH (pH_i), the concentration of other ions (e.g. K^+ , Na^+), ΔpH (difference between pH_o and pH_i), $\Delta\Psi$ (membrane potential) and ΔP (proton-motive force). As external pH approaches that of internal pH, ΔpH is reduced, often with a concomitant rise in $\Delta\Psi$ (assuming ΔP is largely unchanged). Changes in these parameters, then, can influence a host of other processes (e.g. energy coupling, phage adsorption, solute and ion transport, competence, motility) which, in turn, can trigger numerous secondary signals. Primarily for these reasons, we are largely ignorant as to how the signals cascade from the surface. Conversely, some pathways affected by pH may be more easily unravelled. Consider a hypothetical example in which the transport system for a particular metabolite or regulatory molecule (e.g. amino acid, sugar) is affected by external pH. Expression of genes that normally respond to that molecule would then be affected by changes in pH_o because of a change in its intracellular

concentration. In this case the signal transduction pathway would be trivial to discern; its significance, however, would not be so obvious. Coupling a pH response to a particular chemical in the environment may provide an important physiological edge that could allow an organism to successfully compete in a natural environment. To appreciate this perspective one needs only to look at the numerous examples, especially in the field of bacterial virulence, in which a combination of several signals is required to induce a response in the laboratory that begins to mimic that which occurs in nature (DiRita, 1992; Mekalanos, 1992; J. F. Miller *et al.*, 1989; Winans, 1992).

Theoretically, the mechanisms employed by a cell to sense a change in environmental pH can be divided into three broad categories. The first is the direct effects that pH can have on the actual structure or activity of a cellular component. For example, changing the charged state of an amino-acid residue in a protein can have profound effects on the activity of that protein. In such a case, the protein would be serving as a direct sensor of pH. The second mechanism is the ability of the cell to respond to only one particular ionization state of a small molecule. An example of this is the ability of certain weak acids and bases to cross the membrane only in their neutral form. The third mechanism is the ability of cellular components to alter their properties in response to changes in pH-related parameters such as $\Delta\Psi$ and ΔpH . The requirement for sufficient $\Delta\Psi$ for the uptake of aminoglycoside antibiotics serves to illustrate this class (Taber *et al.*, 1987). Membrane proteins are particularly well suited to sensing pH-related signals of all three classes. In particular, proteins spanning the cytoplasmic membrane could 'sense' absolute pH, concentrations of molecules in the environment, and components of ΔP ($\Delta\Psi$ and ΔpH). Several of the genes listed in Table 1 either encode membrane proteins or are regulated by them. While most of those of the regulatory class sense other primary stimuli (none has been shown to sense pH directly), the diversity of processes involved illustrates the concept that pH, like many other environmental signals, probably has subtle effects on numerous pathways.

In general, bacteria have a remarkable capacity to maintain pH_i at a relatively constant value, even as pH_o changes by up to 2 units (Padan *et al.*, 1981). Consequently, it would not be expected that pH_i plays a major role in the signalling pathways for many of the examples listed in Table 1. Of course, there are exceptions. Changes in pH_o can trigger a transient change in pH_i which can subsequently serve as an intracellular signal. Two examples illustrate this point: an *Enterococcus faecalis* proton-translocating ATPase (discussed below) and the *Escherichia coli* SOS response are induced when pH_i is altered as a consequence of external pH changes (Kobayashi *et al.*, 1986; Schuldiner *et al.*, 1986).

Internal pH can also be altered without significantly changing external pH. In their neutral state, membrane-permeable weak acids and bases can diffuse into the cell, but, once dissociated, are unable to diffuse out, resulting in an alteration in pH_i . Ecological and physiological aspects of this aside, this opened the door to experimental assessment of the contributions of pH on either side of the membrane. *E. coli* taxis away from membrane-permeable weak acids and induction of the *E. coli inaA* locus (unknown function) are the result of the cell's ability to sense and respond to changes in intracellular pH (Kihara and Macnab, 1981; Repaske and Adler, 1981; White *et al.*, 1992).

There is a curious example in which the magnitude of an upward pH_o shift, not the absolute value of pH_o or pH_i , has been shown to trigger gene expression. The *E. coli* heat-shock proteins GroE and DnaK were shown to be induced whether cells were shifted from pH_o 6–7.6 or from pH_o 7–8.5 (Taglicht *et al.*, 1987). Two lines of evidence support the notion that ΔpH_i was not responsible for this induction. First, the shift from pH_o 6–7.6 did not alter pH_i . Second, treatment with the weak base diethanolamine, which raised pH_i , did not induce expression. It is somewhat surprising that the relatively mild shift from pH_o 6–7.6 induces some form of a stress response, especially since equally mild downward pH_o shifts do not. The authors suggest that perhaps an abrupt increase in $\Delta\psi$ triggered the response.

Phosphate and proteins involved in its transport and metabolism have been implicated in the ability of *E. coli* to sense changes in pH_o (Rowbury *et al.*, 1992). Mutants lacking the PhoE porin are more acid-resistant while at the same time defective in the ability to induce habituation (a response, induced by moderate acidification, which protects the cell from a more severe drop in pH_o , see below). A model to explain both these phenotypes suggested that H^+ (in some form) gains access to the periplasmic space via PhoE (Rowbury *et al.*, 1992), implicating a role for periplasmic or cytoplasmic membrane components in triggering the habituation response. Consistent with this model is the observation that exogenously added phosphate prevented habituation, presumably because it competed with H^+ entry. Of possible significance to this model is the finding that of 52 proteins induced in *Salmonella typhimurium* by phosphate starvation, 11 were also induced by external acidification (Abshire, 1991).

Proton movement

Four major systems located in the cytoplasmic membrane are responsible for controlling proton flow into and out of the cell: (i) H^+ -coupled ion-transport systems, which exchange ions such as K^+ , Na^+ and H^+ ; (ii) H^+ - F_o/F_1 ATPases, which couple proton movement with the

synthesis and hydrolysis of ATP; (iii) electron transport chains, which are responsible for H^+ efflux; and (iv) transport systems which couple H^+ transport with other solutes. Extensive studies over the past two decades have provided numerous examples in which pH affects the activity of these systems. Because of the requirement for rapid response times, it is probable that the activities of these systems are regulated primarily through conformational changes in their components. However, in a few cases the expression levels of some of these proteins change with pH. This section highlights examples in the first three of these systems.

Na^+/H^+ antiporters

Proper intracellular levels of ions such as K^+ (high) and Na^+ (low) are crucial for proper functioning of intracellular reactions. Consequently, proton-driven antiporters are important for controlling the concentration of these ions in both prokaryotic and eukaryotic organisms (Booth, 1985; Dibrov, 1991; Krulwich *et al.*, 1990; Leblanc *et al.*, 1988; Padan and Schuldiner, 1986; Padan *et al.*, 1981; Pouyssegur *et al.*, 1988). In bacteria Na^+/H^+ antiporters have been implicated in Na^+ -tolerance as well as acidifying the cytoplasm when cells are grown in alkaline conditions (Ishikawa *et al.*, 1987; Krulwich and Guffanti, 1989; McMorro *et al.*, 1989; Padan *et al.*, 1989). The gene (*nhaA*) encoding one of these membrane-bound antiporters (NhaA) from *E. coli* has recently been characterized and shown to be crucial for pH-homeostasis and NaCl tolerance (Padan *et al.*, 1989).

NhaA antiporter function is regulated in two ways. First, its activity is controlled at the protein level. Using purified NhaA reconstituted into proteoliposomes it was found that NhaA activity was stimulated 2000-fold by high pH (Taglicht *et al.*, 1991). Second, expression of *nhaA* increases as NaCl and pH increases (above pH 8.0) (Karpel *et al.*, 1991). Since NhaA apparently senses Na^+ and/or H^+ (reflected in a change in activity as a function of pH and NaCl concentration), it would be a logical candidate to serve as the sensor for initiating the signals which lead to its own increase in synthesis. This possibility, however, was ruled out by finding that an *nhaA-lacZ* fusion was induced in an *nhaA* null mutant. A positive activator of *nhaA* has been identified (NhaR) and found to have homology to the LysR class of bacterial transcriptional activators (Rahav-Manor *et al.*, 1992). A possible role for NhaR in sensing at the membrane was suggested by sequence prediction of two putative transmembrane domains (Rahav-Manor *et al.*, 1992).

H^+ -ATPase

The role of H^+ -ATPases in conferring acid tolerance to

lactococcal, enterococcal and streptococcal species is well documented (Bender *et al.*, 1986; Bender and Marquis, 1987; Booth, 1985; Kinoshita *et al.*, 1984; Konings *et al.*, 1989; Loesche, 1986; Sturr and Marquis, 1990; Sutton *et al.*, 1987; Sutton and Marquis, 1987). As intracellular pH drops, protons are extruded by the F_0F_1 -ATPase (accompanied by the electrogenic uptake of K^+). This system, and its role in pH homeostasis, has been examined in detail in *E. faecalis*. In summary, both increased activity and increased synthesis of H^+ -ATPase is involved in conferring the ability to grow at low pH_o (Kobayashi, 1985; Kobayashi *et al.*, 1984; 1986; Suzuki *et al.*, 1988). Manipulation and measurements of ΔpH , pH_i , pH_o , $\Delta \Psi$ and ATPase level suggested that a decrease in pH_i brought about by external acidification was responsible for the increase in synthesis. To identify the responsible mechanisms, mutants defective in growth at low pH_o were isolated (Suzuki *et al.*, 1988). As expected, one class had lowered activity of the ATPase. Another class, however, had normal activity of the ATPase, but displayed an impaired ability to increase synthesis of the F_1 component as pH_i dropped, thus demonstrating a genetic link between pH homeostasis and regulation of the genes encoding one or all of the F_1 proteins. In this mutant, as pH_i dropped, the membranes became leaky to protons, resulting in a cessation of growth after around 1.5 h. This growth response correlates nicely with the finding that the highest level of ATPase in wild-type cells occurs between 1 and 1.5 h after a fall in pH_o , again linking the expression phenotype with the physiological response. The mutation responsible for the phenotype has not been identified.

Electron transport

Electron transport chains and associated respiratory components constitute the third major system responsible for proton flow; quantitative and qualitative changes in these systems occur with changing growth conditions. Alterations in the type and availability of carbon sources, growth stage, O_2 tension and nature of electron acceptors are a few of the major parameters known to induce these changes (Ingledew and Poole, 1984). The abundance of some of the proteins involved has also been found to vary as a function of pH. These systems constitute another area in which analysis of acidophiles and alkalophiles at the genetic level would be particularly informative, especially since changes in cytochrome content have been observed as a function of pH (Hicks *et al.*, 1991; Ingledew, 1990; Krulwich and Guffanti, 1989; Quirk *et al.*, 1991).

The composition of the electron transport chain in *E. coli* varies as a function of the O_2 level. Under conditions of low O_2 the 'low-affinity' cytochrome *o* oxidase complex (*cyoABCDE*) is repressed and the 'high-affinity' cytochrome *d* system (*cydAB*) is induced (Cotter *et al.*, 1990;

Cotter and Gunsalus, 1992; Fu *et al.*, 1991; Gunsalus, 1992; Ingledew and Poole, 1984; Iuchi *et al.*, 1990; Iuchi and Lin, 1991). Repression of *cyo* is mediated by Fnr and ArcA, two regulators involved in activating and repressing several genes as a function of O_2 (Cotter and Gunsalus, 1992; Cotter *et al.*, 1990; Fu *et al.*, 1991; Gunsalus, 1992; Iuchi *et al.*, 1990). Although the story is incomplete in terms of the mechanisms and signals involved, ArcA- and Fnr-dependent systems independently repress *cyoABCDE*; elimination of either one by mutation results in partial derepression under anaerobic conditions. Mutations in both systems result in further, yet incomplete, repression, suggesting the existence of a third, unidentified, system. The role of pH in regulating this operon has been examined under both aerobic and anaerobic conditions and with *fnr*⁺ and Δfnr strains (Cotter *et al.*, 1990). Under aerobic conditions, when the operon is normally induced, expression varied fourfold between pH values 6.0 and 7.0 (highest at pH 7.0). This pH effect was independent of *fnr*. Under anaerobic conditions, expression was completely repressed between pH values 5.5 and 7.5 in an *fnr*⁺ strain, but in an *fnr* deletion mutant, maximal repression was only observed below pH 6.0. One interpretation of this result is that, under anaerobic conditions, an *fnr*-independent repressing mechanism functions better at low pH. Whether this system is ArcA-dependent or due to a putative third system remains to be resolved.

The process of bacterial sporulation is regulated by a complex set of nutritional and metabolic signals. The level of the electron carrier menaquinone in *B. subtilis* has been suggested to be involved in controlling flow through the electron transport chain during the processes of forespore formation, maturation and germination (Escamilla *et al.*, 1988). Two genes encoding enzymes involved in menaquinone synthesis (*menCD*) have been shown to be transcriptionally regulated during sporulation. One of the signals involved in this regulation is associated with changes in pH of the growth media during the post-exponential phase. Expression is maximal 0.5 h after the onset of stationary phase, followed by a decline over the next 4 h (Hill *et al.*, 1990; P. Miller *et al.*, 1988a,b). This pattern of expression mirrored precisely the changes in pH_o ; pH_o was lowest 0.5 h into stationary phase (probably due to glycolysis and subsequent buildup of acidic end products), then rose over the next 4 h. Moreover, by buffering the media at low or high pH, the transcription pattern could be altered independently of the post-exponential time frame, i.e. expression levels could be sustained at a high level throughout the post-exponential period provided that the pH of the media was kept low. As with the other systems described here, the signal that is being sensed following the change in pH_o has not been identified.

Degradative amino acid decarboxylases

The products of amino acid decarboxylases consist of basic amines (pK_a values around 10). It was suggested as early as 1924 that these products provided an important protective mechanism for bacteria living in low-pH conditions (Hanke and Koessler, 1924). Although *E. coli* contains constitutive biosynthetic arginine and ornithine decarboxylases, these are distinct from the inducible forms which, along with lysine decarboxylase, are maximally produced when cells are grown at low pH, low O₂, and in the presence of substrate (Tabor and Tabor, 1985).

One of these enzymes, lysine decarboxylase, is encoded by *cadA* and, along with *cadB*, forms an operon at minute 93.5 (Auger *et al.*, 1989; Meng and Bennett, 1992a; Tabor *et al.* 1980; Watson *et al.*, 1992). Functional analysis of *cadB*, combined with sequence similarity to other amino acid antiporters, led Meng and Bennett to propose that CadB is a lysine/cadaverine antiporter (cadaverine is formed by the decarboxylation of lysine) (Meng and Bennett, 1992a). Acid-induced transcription of *cadBA* is initiated from a promoter (*Pcad*) located 75bp upstream from the *cadB* initiation codon (Meng and Bennett, 1992b; Watson *et al.*, 1992). Upstream of *cadBA* is *cadC*, which encodes a positive activator of *Pcad* (Watson *et al.*, 1992). Insertion mutations in *cadC* eliminate induction of *Pcad*, but do not affect acid induction of the closely linked gene for arginine decarboxylase (*adi*); thus, CadC is probably not a general global regulator of acid-induced genes (G. N. Bennett, personal communication; Watson *et al.*, 1992).

A model for how CadC activates expression of *cadBA* in response to low pH is based primarily on CadC sequence similarity to OmpR-like transcriptional activators, in particular ToxR from *Vibrio cholerae* (Watson *et al.*, 1992). DNA sequence analysis predicts that CadC is a 512-residue protein with an amino-terminal domain similar to the DNA-binding domain of OmpR, followed by a putative transmembrane and periplasmic domain. In the OmpR-like domain, 12 residues are identical to those found in OmpR from *E. coli*, PhoP from *Bacillus subtilis*, VirG from *Agrobacterium tumefaciens* and ToxR from *V. cholerae* (Melchers *et al.*, 1986; V. L. Miller *et al.*, 1987; Nara *et al.*, 1986; Seki *et al.*, 1987; Wurtzel *et al.*, 1982). Unlike the other members of this class of proteins, however, CadC and ToxR do not possess the phosphorylation domain common to the response proteins found in the histidine kinase sensor–regulator systems.

Like other promoters that are positively regulated, *Pcad* has a sigma-70 consensus –10 sequence, but a poor –35 region. Mutational and functional analysis of the region upstream of *Pcad* revealed that a sequence at –120bp is required for activation (Meng and Bennett, 1992b; Watson *et al.*, 1992). The ability of a 66bp fragment containing this site, when placed on a high-copy-number plasmid, to

compete for a factor required for *Pcad* activation, combined with *in vivo* footprint data, suggests that this sequence is part of a binding site (Meng and Bennett, 1992b; Watson *et al.*, 1992). However, there is no direct evidence that CadC binds to this sequence. Interestingly, part of this sequence is also located upstream from *lysU* (see below), which is also induced by growth at low pH (Lévêque *et al.*, 1991). Expression of *cadC* appears to be constitutive, implying that the activity of CadC changes in response to a signal generated from a drop in pH_o. In its simplest form the model proposes that CadC undergoes a conformational change at low pH_o which, in turn, alters the ability of the amino-terminal cytoplasmic domain to function as a transcriptional activator at *Pcad* (Watson *et al.*, 1992).

Recently it has been shown that the DNA-binding protein H-NS (encoded by *osmZ/hns/bglY/drdX*) is involved in repression of *cadBA* and *adi* (the operon encoding arginine decarboxylase) at neutral pH (G. N. Bennett, personal communication). This abundant nucleoid protein has been implicated in regulating (both positively and negatively) the expression of genes involved in the cell's response to many different environmental signals (Bhriain *et al.*, 1989; Higgins *et al.*, 1990); pH can now be added to that list. Models for H-NS action centre on its possible effects on DNA topology, which, in turn, could affect promoter activity by a number of possible mechanisms (Higgins *et al.*, 1990). The specific mechanism by which *cadBA* and *adi* are affected remains to be elucidated.

The products of two other genes, *lysU* and *lysP* (also called *cadR*), may also participate in this lysine degradative pathway. Expression of *lysU*, which encodes a lysyl-tRNA synthetase, and *lysP*, which encodes a lysine transport protein, can be induced by the same conditions that induce *cadBA* (Hassani *et al.*, 1991; Hickey and Hirshfield, 1990; Lévêque *et al.*, 1991; Steffes *et al.*, 1992; VanBogelen *et al.*, 1983). In view of the fact that CadB, LysP and probably CadC are membrane proteins, one wonders if there are interactions between any of these which serve to regulate their activities, especially in the light of the observation that mutations in *lysP* affect expression of *cadA* (Tabor *et al.*, 1980).

Culture conditions have a marked effect on the concentration of putrescine and spermidine in *E. coli* (Tabor and Tabor, 1985). Three of these conditions, pH, O₂ level, and the presence of lysine or arginine, are identical to those that regulate expression of *cadBA* and *adi*, suggesting that these genes influence cellular polyamine content. Together with the observation that about 10% of the cellular spermidine, putrescine and cadaverine is found in the cell envelope of *E. coli* and *S. typhimurium* (Koski and Vaara, 1991), the question is raised as to whether a qualitative or quantitative change in association of poly-

amines with cell surface components is one of the cellular responses to changes in pH_o . Consistent with such a hypothesis is the observation that the makeup of polyamines changes in alkalophilic bacilli as a function of pH (Kroll, 1990). A more thorough analysis of the role of *cadBA*, *adi* and their products with respect to polyamine concentrations and response to pH needs to be carried out.

Adaptation/habitation to low pH

Exposure of *E. coli* and *S. typhimurium* to moderately low pH_o (pH 5.5–6.0) induces a process that protects the cell from a subsequent challenge at lower pH_o (pH 3.4–4.0) (Foster, 1992; Foster and Hall, 1990; 1991; Raja *et al.*, 1991a). This response, called the acid tolerance response (ATR) in *S. typhimurium* and habituation in *E. coli*, is characterized by the requirement for protein synthesis during the moderate pH treatment. The purpose of this preshock treatment appears to be to induce a system(s) which prevents a lethal drop of internal pH. Since there are several recent publications describing various aspects of this work (Foster, 1991; 1992; Foster and Aliabadi, 1989; Foster and Hall, 1990; 1991; 1992; Goodson and Rowbury, 1991; Hickey and Hirshfield, 1990; Raja *et al.*, 1991a,b; Rowbury *et al.*, 1992), I will only summarize what is known in terms of the pattern of gene expression during acid treatments of *E. coli* and *S. typhimurium*.

As shown by two-dimensional (2-D) gel analysis of proteins synthesized by *S. typhimurium* during the ATR (shift from pH_o 7.6 to 5.8), the synthesis level of at least 18 proteins is altered (12 increased and 6 decreased) (Foster and Hall, 1990). Although it is not clear which of these are actually required for the adaptive response, the analysis of mutants provides good evidence that at least some are involved. An acid-resistant mutant (carrying the *atr-1* allele) showed increased expression of six of the 18 proteins along with higher expression of five other proteins. Likewise, acid-sensitive mutants displayed altered expression of several ATR proteins. Of particular interest is the finding that *fur* mutants (ferric uptake regulator, encoding a regulator of genes involved in iron uptake and utilization), displayed altered expression of eight of the 18 ATR proteins and are unable to induce the ATR (Foster and Hall, 1990; 1992).

Habitation to acidic conditions in *E. coli* appears to be very similar to the ATR of *S. typhimurium*; a protein synthesis-dependent process occurs at pH_o 5.0–6.0 that allows the cells to survive more acidic conditions (Raja *et al.*, 1991a). As in *S. typhimurium*, most of the proteins induced in this response have not been identified, nor is it known which ones are required for the response. Properties of habituated cells, however, provide clues as to the nature of the induced proteins. For example,

habituated cells more efficiently repair DNA damaged as a result of exposure of cells to low pH_o , as well as becoming more resistant to ultraviolet irradiation (Goodson and Rowbury, 1991; Raja *et al.*, 1991b). This resistance was shown to be independent of *recA* and *uvrA*, genes which are involved in the SOS response. Interestingly, a neutral to alkaline shift in pH_o , which results in a transient increase in *E. coli* pH_i (Schuldiner *et al.*, 1986), also increases ultraviolet resistance; however, in contrast to that induced by acid habituation, this response is primarily RecA-dependent and typical of an SOS response (i.e. filamentation, induction of *uvrA*, LexA-dependent, transient).

Several reports, based on protein gel analyses and gene/operon fusions, have provided additional examples of genes induced or repressed during various pH treatments, although most of these genes have not been implicated in adaptation or habituation; in fact, the functions of most are unknown (Abshire, 1991; Aliabadi *et al.*, 1988; Bingham *et al.*, 1990; Foster, 1991; Foster and Hall, 1990; Heyde *et al.*, 1991; Heyde and Portalier, 1987; 1990; Hickey and Hirshfield, 1990; Slonczewski *et al.*, 1987; Taglicht *et al.*, 1987; White *et al.*, 1992). Nevertheless, two trends have emerged from these reports. First, expression of several membrane proteins is affected by acid treatment. This is not unexpected since these proteins would be expected to be exposed to the extracellular pH and/or involved in processes associated with proton movement. Adjusting the level of these proteins is probably required to compensate for changes in activities brought about by changes in pH_o . The second trend is that treatments that decrease pH_i result in the induction of proteins known to be induced by other stresses. For example, shifting *E. coli* from pH_o 6.9 to pH_o 4.3, a treatment that overwhelmed the pH homeostatic mechanisms, induced the heat-shock proteins, DnaK, GroEL, HtpG and HtpM (Heyde and Portalier, 1990), while a more moderate treatment (pH_o 7.0 to pH_o 5.0) did not (Hickey and Hirshfield, 1990). Similar results were observed with *S. typhimurium* in which acid-shock treatments induced DnaK, GroEL, GroES and GrpE (Abshire, 1991; Foster, 1991). These results suggest that these treatments result in damage to intracellular proteins.

Regulation by pH and O_2

It is impossible to ignore the numerous examples in which both pH and O_2 levels have been shown to affect the expression level of a particular gene. Two-dimensional gel analysis of proteins expressed during *S. typhimurium*'s response to pH and O_2 levels revealed several overlaps, including *aniG*, *hyd*, *ompF*, *ompC*, as well as other ATR and acid-shock proteins (Aliabadi *et al.*, 1988; Bhriain *et al.*, 1989; Foster and Aliabadi, 1989; Foster and Hall, 1991; Hickey and Hirshfield, 1990; Higgins *et al.*, 1990; Spector *et al.*, 1986). In *E. coli*, expression of *cadBA*, *lysU*, *lysP*,

cyo, *cyd*, *ompF*, *adi*, *speF*, *aceF* and four unmapped *phoA* gene fusions is also regulated by these two signals (Auger and Bennett, 1989; Cotter *et al.*, 1990; Heyde *et al.*, 1991; Heyde and Portalier, 1987; Hickey and Hirshfield, 1990; Kashiwagi *et al.*, 1991; L  v  que *et al.*, 1991; Meng and Bennett, 1992b; Steffes *et al.*, 1992; Tabor and Tabor, 1985; Watson *et al.*, 1992).

Three global regulators in *E. coli* have been identified which control the expression of genes according to the level of oxygen or presence of alternate electron acceptors: ArcB/ArcA, NarX/NarL and Fnr (Gunsalus, 1992; Iuchi and Lin, 1991). Since the major cellular response to changing oxygen tension is an alteration in respiration or fermentation, most of the operons regulated by these three systems encode proteins involved in these processes (e.g. dehydrogenases, reductases, cytochromes, TCA cycle enzymes). The Arc system is primarily responsible for repressing, during anaerobic growth, genes involved in aerobic metabolism. NarX/NarL- and Fnr-regulated genes encode functions involved in utilizing the alternate electron acceptors nitrate and fumarate. Except for limited data with *cadBA*, *cyo* and *cyd*, however, little is known of the extent to which these systems respond to changes in pH (Auger and Bennett, 1989; Cotter *et al.*, 1990).

Where in the regulatory networks do the O₂ and pH signals intersect? In the case of *cadBA* we know that low O₂ tension by itself does not result in induction. It follows from this that since *cadC* is required for pH induction, it should also be required for the O₂ effect. This is indeed the case (Watson *et al.*, 1992). Thus, low O₂ apparently accentuates the pH effect through the action of CadC. It is instructive to compare this situation with what has been observed with the acid-induced *aniG* locus of *S. typhimurium* (Foster and Aliabadi, 1989). Like *cadBA*, *aniG* expression is induced an additional two- to fourfold by anaerobiosis. Under alkaline conditions *aniG* is repressed by the *earA* gene product through its binding to the *aniG* operator/promoter. Upon acidification, EarA-mediated repression is relieved. In contrast to *cadBA*, however, the O₂ effect can be uncoupled from EarA-mediated pH regulation. Mutants of *earA* that relieve the requirement for acid induction still show a two- to fourfold induction by anaerobiosis. If anaerobiosis was facilitating derepression by EarA, one would have expected the opposite result. Thus, the pH-activating mechanism appears to be separate from the O₂ effector. It will be interesting to see whether mutants that are derepressed for *cadBA* at neutral pH still display an O₂ effect.

Microbial–host interactions

Bacteria infecting plants and animals respond to a myriad of chemical and physicochemical signals which, in many

cases, results in the induction or repression of ‘virulence’ genes. As one might expect, pH appears to be one of the signals involved in some of these responses. To what extent, if any, the products of these ‘virulence’ genes are actually involved in regulating pH homeostasis or pH adaptive responses is unclear. It is likely that in many cases the bacteria use the characteristic pH of a host micro-environment as one of many signals responsible for triggering the full repertoire of appropriate genes (Brewin, 1991; Caetano and Gresshoff, 1991; DiRita, 1992; Long, 1989; Mekalanos, 1992; J. F. Miller *et al.*, 1989; Rahme *et al.*, 1992; Winans, 1992).

Agrobacterium vir genes

The ability of *Agrobacterium* to form a tumour and transfer DNA to its host plant is dependent upon expression of *vir* genes localized on the Ti plasmid. Transcriptional regulation of these operons is controlled primarily through the action of the histidine-kinase sensor/regulator proteins VirA and VirG, respectively. The activities of these proteins, in turn, are controlled by environmental signals including chemical signals (e.g. phenolic compounds and monosaccharides released from the plants), temperature and external acidification (Winans, 1992).

Two distinct pH-mediated responses of *vir* gene induction have been observed: VirA/VirG-independent transcription of *virG*, and VirA/VirG-dependent transcription of *vir* operons are maximal at low pH_o (Chen and Winans, 1991; Mantis and Winans, 1992; Stachel *et al.*, 1986; Winans, 1990; Winans *et al.*, 1988). The former response appears to represent an early stage (‘pump-priming’) in the *vir* gene induction process, responsible for increasing the level of VirG which, in turn, further activates its own transcription as well as the other *vir* promoters in a VirA/VirG-dependent manner. However, induction of *virG* expression is not the only role for pH since expression of *virG* from *Plac* is not sufficient to induce the other *vir* promoters; low pH is also required (Chen and Winans, 1991). Although the mechanism for this pH response is not understood, it is, at least in part, mediated by VirA (Turk *et al.*, 1991). A deletion of the periplasmic domain of VirA resulted in the inability to respond to pH_o, but did not affect activation by chemical stimuli (Melchers *et al.*, 1989).

Salmonella

The *S. typhimurium* PhoP/PhoQ system regulates expression of genes implicated in pathogenesis and survival within macrophages (Miller, 1991; S. I. Miller *et al.*, 1989). Transcription of at least three of these genes, *pagA*, *pagB* and *pagC*, increases within macrophages coincidentally with vacuole acidification (around pH 5.0), suggesting that

pH is one of the signals triggering PhoP-mediated activation (S. I. Miller, personal communication; Abshire, 1991). Moreover, treatment of macrophages with a weak base, which inhibits internal acidification, prevents induction of these genes. As one might expect, signals other than low pH_o are involved; growth in Luria broth (LB) at pH 5.0 results in weak induction relative to that seen within macrophages. Not only does *S. typhimurium* appear to respond to vacuolar pH, but its presence delays acidification of the vacuole itself, resulting in a delay in expression until several hours following infection (S. I. Miller, personal communication). The combined approaches of identifying the proteins induced by acidification, examining the pattern of gene expression in tissue-culture cells, and determining the role of these genes in animal infection models offer a particularly exciting prospect for unravelling an important pH-mediated response.

Vibrio cholerae

The ToxR regulon of *V. cholerae* consists of several different genes, many of which are associated with virulence. Included are those encoding cholera toxin (*ctxAB*), production of the TCP pilus (*tcp*), outer membrane proteins OmpU and OmpT, accessory colonization factor (*acf*), aldehyde dehydrogenase (*aldA*) and several others identified by *phoA* fusions (*tag*) (Parsot and Mekalanos, 1991; Peterson and Mekalanos, 1988). ToxR-mediated induction involves different environmental signals as well as other regulatory proteins (e.g. ToxT, ToxS) (DiRita, 1992). Growth at low pH_o is also an obligatory signal; induction is observed in media starting out at pH 6.5 but not at pH 8.4. The mechanism underlying this pH effect is not known. A more complete analysis of the role of pH, especially with regard to which of the other environmental signals or regulatory proteins are required, is eagerly awaited.

Closing comments

For the most part, our understanding of environmentally induced changes in gene expression, at least for *E. coli* and *S. typhimurium*, has left the arena of identifying operon-specific regulators. The major elements mediating many of these responses have now been identified; not so with pH. Fortunately, there are numerous exciting paradigms under current investigation which should provide us with a better understanding of how the cell senses pH and how those signals are transmitted to the proteins that transcribe and translate genetic material. Consideration of the number and diversity of physiological and metabolic parameters affected by pH suggests that in many cases pH effects are secondary. Further investigation of systems in which pH appears to be a primary signal and where

regulatory proteins have been identified (e.g. NhaR, CadC and EarA) should be especially valuable.

Notes added in proof

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