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Topical Review

Intracellular pH and Membrane Potential as Regulators in the Prokaryotic Cell

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I. Proton Current of Bacteria

Circulation of protons across the plasma membrane plays a primary role in energy transduction of the bacterial cell (Mitchell, 1966, 1979; Harold, 1982, 1986). The respiratory and photosynthetic electron transport chain as well as the H^+/ATPase and bacteriorodopsin mediate electrogenic transport of protons outwards, maintaining an electrochemical gradient of protons, $\Delta\tilde{\mu}_{H^+}$,* across the membrane. The proton current loop is completed by many different molecular devices that allow protons to return to the cytoplasm while performing useful work at the expense of the $\Delta\tilde{\mu}_{H^+}$. These reactions include oxidative phosphorylation and photosynthetic phosphorylation, transhydrogenation of pyridine nucleotides, motility and active transport.

Energy conversions coupled to the proton current have been extensively reviewed (Harold, 1982, 1986; Mitchell, 1966, 1979). The present essay will focus on another aspect of the proton gradient which is not its obvious functions as a driving force. This is the regulatory role played by the proton current parameters in the prokaryotic cell.

The proton circulation parameters include the $\Delta\tilde{\mu}_{H^+}$ [Eq. (1)] and its components $\Delta\psi$ and ΔpH. The latter in the prokaryotic cell is the difference between the cytoplasmic pH (pH_i) and the pH of the bacterial environment (pH_o).

$$\frac{\Delta\tilde{\mu}_{H^+}}{F} = \Delta\psi - 2.3\frac{RT}{F}\Delta pH \qquad (1)$$

(R, gas constant; T, absolute temperature; F, Faraday constant).

II. Growth of Bacteria without an Electrochemical Proton Gradient

Since the proton current is involved in so many energy conversion processes, design of an experiment that will allow differentiation between the energetic role of the proton current and additional independent roles is not simple. It is easy to dissipate the proton gradient by short circuiting the proton current with H^+-ionophores and study the effect of this manipulation on any particular reaction. However, dissipation of the proton gradient will hamper many energy transducing and dependent processes required for cell viability, such as synthesis of ATP and the active transport of ions and essential organic substrates. Therefore, an observed effect caused by inhibition of the proton gradient on a particular reaction may be indirect and, therefore, misleading.

Realizing this complication, Harold and Brunt (1977) utilized *Streptococcus faecalis* for a very elegant experiment to determine whether proton circulation in bacteria is involved, beyond its energetic role, in DNA replication, RNA and protein synthesis, cell growth and cell division. *Streptococcus faecalis* is an anaerobic fermentative organism that glycolyticaly synthesizes ATP and normally maintains a circulation of protons across the membrane by means of the H^+/ATPase. The proton current was short circuited by gramicidin and other H^+ ionophores, and the effect on cell growth was stud-

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* *Abbreviations:* $\Delta\tilde{\mu}_{H^+}$, proton electrochemical gradient, $\Delta\tilde{\mu}_{H^+} = \Delta\psi - 2.3\ RT/F\ \Delta$ pH R, gas constant; T, absolute temperature, F, Faraday. $\Delta\psi$, electrical potential; Δ pH, $pH_i - pH_o$. pH_i, cytoplasmic pH; pH_o, extracellular pH. CCCP, Carbonyl cyanide *m*-chlorophenyl-hydrazone. FCCP, Carbonyl cyanide-*p*-trifluoromethyoxyphenylhydrazone. EDTA, ethylenediaminetetra-acetic acid. DNP, dinitrophenol. hsps, heat shock proteins.

ied under different incubation conditions. It was found that *S. faecalis* grew normally in the absence of the proton current as long as the growth medium was buffered at pH 7.5–8.2, contained 2 mM of each amino acid, high K^+ (0.28–1 M) and low Na^+ (less than 0.01 M) concentrations. These strict growth requirements in the presence of gramicidin, which compensate for the lack of the proton gradient, reflect the known roles of this gradient in the normal cell: It is essential to drive synthesis of ATP, if it is not supplied glycolyticaly or by other substrate level phosphorylations; it is essential to drive uptake of metabolites (Kaback, 1983), establish K^+ and Na^+ gradients across the membrane and maintain intracellular pH homeostasis (Mitchell, 1966; Harold & Papineau, 1972*a*,*b*; Padan, Zilberstein & Rottenberg, 1976; Padan, Zilberstein & Schuldiner, 1981; Harold, 1982; Cobley & Cox, 1983; Krulwich & Guffanti, 1983; Booth, 1985).

The growth of *S. faecalis* without a proton gradient also shows that the proton electrochemical gradient is dispensable for the biosynthesis and replication of this bacterial cell. Similarly, Kopecky, Copeland and Lusk (1975) showed that *Escherichia coli* "killed" by colicin K grow when supplied with excess K^+ and Mg^+. Also, *E. coli* cells can grow in the presence of the protonophore, CCCP, when glucose is used as an energy source, at pH 7.5 (Kinoshita, Unemoto & Kobayashi, 1984). It is suggested that the conclusion reached with *S. faecalis* might be generally applicable to all prokaryotes.

By contrast, there is mounting evidence that in many different bacteria there are specific reactions that are somehow modulated by the proton concentrations or by membrane potential, in a manner unrelated to their energetic role. Unfortunately, however, Harold and Brunt-type experiment (Harold & Brunt, 1977) have not been conducted in any of these systems.

III. Experimental System to Study Regulatory Roles of $\Delta\psi$ and ΔpH

The experimental system of *E. coli* frequently used to test the regulatory roles of the proton current consisted of washed resting cells harvested at the logarithmic phase of growth and incubated in defined buffers and salts solution under nongrowing conditions. These cells were also often treated with EDTA to render them sensitive to ionophores, allowing the simultaneous manipulation and measurement of ΔpH and $\Delta\psi$ (Padan et al., 1976; Zilberstein, Schuldiner & Padan, 1979; Kashket, 1981, 1985). EDTA-treated and nontreated resting cells, like growing *E. coli* cells, are viable, synthesize macromolecules and maintain their intracellular pH constant by modulation of ΔpH and $\Delta\psi$ over a wide range of extracellular pH (pH 6–8) (Padan et al., 1976, 1981; Zilberstein et al., 1979; Kashket, 1985). Their ΔpH, which is 1.8 at pH 6, decreases progressively with pH_o so that it is zero at pH 7.8 and becomes negative as the cytoplasm becomes acidic, relative to the medium, at alkaline pH. $\Delta\psi$ increases in a compensatory fashion up to pH_o 8. Therefore, cells at acidic pH provide an experimental system with different magnitudes of both $\Delta\psi$ and ΔpH. Cells at pH_o 7.8 provide an experimental system with only $\Delta\psi$. Above the later pH_o, $\Delta\psi$ and negative ΔpH exist across the membrane. Determination of pH profiles of different processes were used to study the requirements of a process for either $\Delta\psi$ and/or ΔpH.

Since EDTA-treated cells are sensitive to ionophores, it is possible to differentially collapse either ΔpH or $\Delta\psi$ (Padan et al., 1976). Usually valinomycin (2 μg/mg cell protein) in the presence of K^+ at 10–100 mM is used to dissipate $\Delta\psi$ providing cells with only ΔpH. Nigericin (2 μg/mg cell protein) is usually used to collapse ΔpH providing cells with only $\Delta\psi$ (Santos & Kaback, 1981). These cells were used to find the dependence of a particular process on these parameters of the proton current. Utilizing EDTA-treated cells, it is also possible to quantitate $\Delta\psi$ and ΔpH (Kashket, 1985) and, therefore, to monitor the effect of any particular reaction on $\Delta\psi$ or ΔpH. Hyperpolarization as well as depolarization can thus be observed. Other neutrophilic bacteria are very similar to *E. coli* with respect to the proton current and its manipulation (Padan et al., 1981).

Acidophilic and alkalophilic bacteria exhibit exaggeration of the pattern of the proton current observed in *E. coli* at acidic or alkaline pH, respectively. Therefore, they can be manipulated in a similar fashion with respect to ΔpH and $\Delta\psi$ (Guffanti et al., 1978; Delmer, Benziman & Padan, 1982).

It should be emphasized that the data indicating a regulatory role of the proton current must be carefully analyzed. Nonenergetic involvement of the proton circulation can be implied only from experiments in which care is taken to verify that the ATP level, pH_i and viability of the cell are normally maintained.

IV. Regulatory Role of $\Delta\psi$

A. Requirement for $\Delta\psi$ for Cellulose Synthesis in *Acetobacter xylinum*

Acetobacter xylinum is a gram negative bacterium which synthesizes abundant quantities of cellulose

at the cytoplasmic membrane that accumulates as an extracellular pellicle. As such it provides a good experimental system to study cellulose synthesis and factors affecting it.

Stimulated by observations obtained with cotton fibers (Carpita & Delmer, 1980), Delmer et al. (1982) tested whether $\Delta\psi$ modulates cellulose biosynthesis in *A. xylinum*. They used EDTA/valinomycin treatment to render metabolizing cells freely permeable to K^+. The intracellular pH of this bacterium is about 6. At an extracellular pH of 6, in the presence of low K^+ (1 mM), little or no ΔpH but substantial $\Delta\psi$ (75 mV) existed across the membrane. Under these conditions cellulose synthesis proceeded at high rates showing that ΔpH is not required for this process. Dissipation of $\Delta\psi$ accompanied by compensatory increase in ΔpH was achieved by increasing K^+ concentration to 50–100 mM and decreasing the extracellular pH to 4.2. This manipulation resulted in severe inhibition of cellulose synthesis. The effect was reversed by restoration of $\Delta\psi$ when the cells were washed and incubated at low K^+.

Direct inhibition by the high extracellular K^+, which is unrelated to changes in the $\Delta\psi$ seemed unlikely, because cells incubated in high K^+ medium in the absence of valinomycin show no inhibition and high K^+, 100 mM, in the presence of valinomycin did not inhibit in vitro cellulose synthesis. Under the inhibitory conditions, lack of $\Delta\psi$ may affect intracellular concentration of different ions which in turn may change the rate of cellulose synthesis. Change of Na^+ concentration up to 100 mM had no effect both in the presence or absence of valinomycin. Mg^{2+} was added at 5–10 mM to all reaction mixtures. Leak of essential ions in the absence of $\Delta\psi$ cannot be ruled out. However, under the inhibitory conditions no substantial impairment of energy metabolism occurred. The ΔpH was of sufficient magnitude to sustain normal rate of active glucose transport allowing normal CO_2 evolution in this obligate aerobic organism. Intracellular pH was maintained at pH 5.8–6 throughout the experiment, which is in the range of the physiological pH_i of acidophiles (Padan et al., 1981). Taken together these results suggest that for cellulose synthesis in vivo there may be a requirement for $\Delta\psi$ which is independent of its energetic role. Recent experiments support the conclusion since a soluble in vitro system which synthesizes cellulose without $\Delta\psi$ has been purified from the cells (Aloni et al., 1983).

B. Involvement of $\Delta\psi$ in Penetration of DNA into the Bacterial Cell

The penetration of DNA into bacteria via conjugation (Grinius & Berzinskiene, 1976), phage infection (Labedan & Goldberg, 1979; Grinius, 1980; Wagner, Ponta & Sweiger, 1980) and transformation (Grinius, 1980; Santos & Kaback, 1981) have been shown to depend on the presence of $\Delta\mu_{H^+}$ across the bacterial cytoplasmic membrane. As will be summarized, below, in the case of phage infection and plasmid transformation, this requirement appears to stem from a regulatory role played by $\Delta\psi$ in DNA penetration.

1. Involvement of $\Delta\psi$ in Ca^{++} Induced Plasmid Transformation

Study of the involvement of $\Delta\mu_{H^+}$ in plasmid transformation was carried out in *E. coli* cells treated with EDTA. The cells "sensitized" to ionophores were transformed with plasmid under various conditions (Santos & Kaback, 1981), then washed to remove the ionophores and grown on solid medium under plasmid-selective as well as under plasmid-nonselective growth conditions. Transformation efficiency was calculated from the difference in the number of colonies appearing under both conditions. When transformation was studied as a function of ambient pH, the efficiency of the process varied with pH in a manner similar to $\Delta\psi$ and not to ΔpH. Addition of valinomycin in the presence of K^+ reduced $\Delta\psi$ as previously shown (Padan et al., 1976) and concomitantly decreased transformation efficiency (Santos & Kaback, 1981). In the presence of nigericin ΔpH was collapsed with hardly any effect on transformation.

Transformation efficiency but not viability of the cells was affected by treatment with FCCP or DNP which totally collapsed the $\Delta\tilde{\mu}_{H^+}$ during transformation. Therefore, it was concluded that the membrane potential plays a critical role in Ca^{2+}-induced transformation which is not related to its energetic role. Preliminary observations provided tentative indication that $\Delta\psi$ may be involved primarily in binding of DNA to the cell surface rather than in translocation through the membrane (Santos & Kaback, 1981).

2. Involvement of $\Delta\psi$ in Initial Steps of Phage Infection

a. Phage Adsorption Induces Depolarization of the Membrane. Adsorption of three different phages, T4, T5 and BF23, induce transient depolarization of $\Delta\psi$ (Letellier & Labedan, 1985). They also induce an immediate release of respiratory control of starved *E. coli* cells (Kalasauskaite & Grinius, 1979; Kalasauskaite et al., 1983; Letellier & Labedan, 1985). Both these phenomena were induced by T4

ghosts devoid of DNA and internal proteins (Letellier & Labedan, 1985). Since the effect was similar in magnitude to proton ionophores, it was suggested that phage adsorption induces an entry of protons into the cytoplasm. The release of respiratory control and depolarization was dependent on envelope-bound Ca^{2+} in the case of T4, but neither T5 nor BF23 required Ca^{2+}. These results led to the suggestion that phages which adsorb to lipopolysaccharides (T4) have a calcium-dependent transmission of a signal for membrane depolarization and phages which adsorb to outer membrane proteins (T5 or BF23) have a direct, calcium-independent signal transmission (Letellier & Labedan, 1985).

Recently it has been shown that bacteriophage Lambda binds to unilamellar liposomes bearing the receptor protein, LamB derived from *Shigella* (Roessner & Ihler, 1986). Transmembrane channels are formed through which the DNA is injected into the internal aqueous space of the liposomes and permit the entry and escape of small molecules but not of proteins. The channels are stable and persist after DNA injection. In this artificial system it appears that adsorption channel formation and DNA injection are coupled (Roessner & Ihler, 1986).

Numerous studies (Ponta et al., 1976; Wilson, 1982; Kewelch & Bakker, 1984) indicate that ions and small molecules leak from the cells for a short time following DNA entry. These results suggest that the DNA molecules enter the bacterial cells through transmembrane channels in the membrane.

b. $\Delta\psi$ is Required for T4 Phage Injection. The injection of T4 DNA into *E. coli* cells was inhibited by incubating the cells at low temperatures or by addition of proton ionophores at 37°C, suggesting that membrane energization is required for DNA injection (Labedan & Goldberg, 1979). In order to investigate the involvement of the proton current in the injection process, EDTA-treated *E. coli* cells were incubated in pH-buffered nutrient broth. These cells allow normal adsorption, injection and growth of the bacteriophage. There was no effect of extracellular pH on the injection process between pH_o 6 and 8. Since over this pH_o range ΔpH decreases from 1.8 at pH 6 to 0 at pH 7.8, it was deduced that ΔpH is not required for the process. Accordingly, nigericin had very little effect on the process both at pH_o 6 and 7.4. These results show that the injection is also independent of pH_i at the range 6–7.4.

Addition of $TPMP^+$ (1 mM), a permeable cation, colicin K (pH_o 6–7) or valinomycin in the presence of 40 mM K^+, all of which collapse $\Delta\psi$, led to marked inhibition of injection. When *unc*$^-$ cells were used in the experiment, it was shown that the level of ATP was not changed during valinomycin/K^+ treatment. Taken together these results suggested that $\Delta\psi$ is required for injection. The injection process was also measured as a function of different $\Delta\psi$ values obtained at different concentrations of CCCP or potassium in the presence of valinomycin (Labedan et al., 1980). A threshold of 60–110 mV $\Delta\psi$ was required for injection over the pH_o range of 6–8.

Irreversible adsorption, unplugging and initial exposure of the DNA terminus occurred at 4 or at 37°C in the presence of $\Delta\psi$-collapsing conditions, implying that these initial steps of phage infection do not require $\Delta\psi$. The injection process into the cytoplasm was inhibited by the ionophores, in a reversible fashion, meaning that the DNA was stopped at the $\Delta\psi$-requiring step, without any damage to it or to other phage or bacterial components. Since the phage DNA in the ionophore-poisoned complex was unavailable to DNAase even after permeabilization of the cells, it was concluded that the DNA is blocked after being channeled to the cytoplasmic membrane but before any strong interaction occurs with the membrane (Labedan & Goldberg, 1979).

As mentioned above (IVB.2; Kalasauskaite et al., 1983), it was shown that T4 phage infection released respiratory control of starved, infected cells; therefore, it was suggested that $\Delta\psi$ is required to drive DNA transport. However, since it was shown that the "uncoupling" effect of the phages can be obtained with phage ghosts devoid of DNA, it was concluded that the depolarization effect is unrelated to phage injection (Letellier & Labedan, 1985). It, therefore, appears that during injection of T4 DNA, $\Delta\psi$ has a regulatory role and is not a motive force of injection (Labedan et al., 1980; Letellier & Labedan, 1985).

V. The Proton Current across the Membrane is Directly Involved in Regulation of Cytoplasmic pH

Bacteria live in aquatic habitats that differ markedly in pH_o, from pH 2 of acidic springs to pH 11.5 of soda lakes. Bacteria can encounter temporal and drastic fluctuations in their environmental pH_o, that can reach 2–3 pH units (Padan et al., 1981).

All bacteria adapt to their pH_o conditions, by a very efficient homeostatic mechanism that maintains a constant intracellular pH. A mechanism based on the modulation of the ΔpH and $\Delta\psi$ created by the primary proton pumps of the cells, i.e., electron transport-linked pumps and/or H^+/ATPase (Padan et al., 1981; Cobley & Cox, 1983; Krulwich & Guffanti, 1983; Booth, 1985) appears to be com-

mon to many bacterial species. The ΔpH—basic inside at low and neutral pH_o (pH 2–7.8)—decreases with increasing pH_o so that the intracellular pH is kept constant at around pH 6.5 in the acidophiles (Cobley & Cox, 1983) and at pH 7.6 in the neutrophiles (Padan et al., 1981). The ΔpH of neutrophiles and alkalophiles even reverses orientation and becomes negative (i.e., acidic inside) at alkaline pH_o (above 7.8 for neutrophiles and above 8.5 for alkalophiles) (Padan et al., 1981). pH_i of the alkalophiles is poised at 8.5 up to pH_o of 11.5 (Krulwich & Guffanti, 1983).

The $\Delta\psi$ increases with pH in a compensatory fashion relative to the ΔpH, being 0 at pH_o 2 in the acidophiles and increasing to about 60 mV at pH_o of 5. In the neutrophiles it further increases to 120–160 mV at pH_o 8 (Felle et al., 1980; Zilberstein et al., 1979) which is also the maximal values of the alkalophiles (Padan et al., 1981).

Due to the specific modulation in its parameters, the $\Delta\tilde{\mu}_{H^+}$ is high in the acidophiles and in the neutrophiles. In the latter case it is constant over a pH_o range of 6–8.5 (Zilberstein et al., 1984). Thus pH homeostasis is achieved in these bacteria together with bioenergetic homeostasis. In the alkalophiles most of the $\Delta\tilde{\mu}_{H^+}$ is sacrificed for pH homeostasis; it becomes very small due to the acid inside [i.e., ΔpH becomes *negative—see* Eq. (1)] (Krulwich & Guffanti, 1983).

The mechanism of modulation of the primary proton pumps is as yet unknown. In the acidic range it appears that K^+ transport is involved (Bakker & Mangerich, 1981; Tokuda, Nakamura & Unemoto, Booth, 1985). In the basic range both a Na^+/H^+ and a K^+/H^+ antiporter have been implied (Padan et al., 1981; Krulwich & Guffanti, 1983; Nakamura, Tokuda & Unemoto, 1984; Booth, 1985).

In view of the variation of ΔpH with pH_o, which characterizes all known bacteria, it is not surprising that, as yet, no regulatory role has been claimed for the ΔpH other than its central role in pH homeostasis. In contrast, regulatory effects have been ascribed to pH_o and pH_i, both in pH homeostasis as well as in other processes.

VI. pH as a Regulatory Signal in General and in pH Homeostasis

The concentration of protons plays a fundamental role in essential physicochemical reactions. Accordingly, most proteins and other biologically important molecules have a narrow pH range of optimum activity and stability. In most cases the pH optimum falls around neutrality even for molecules isolated from organisms living in extreme pH environments. It is not surprising, therefore, that intracellular pH is maintained under tight control.

It should be emphasized that since pH is so intensively involved in biochemical processes, the existence of a correlation between a change in pH and a particular process does not necessarily mean that the pH is a regulator. Rather than triggering the particular process, it may be an accompanying related or unrelated event. Realizing this limitation, we will define what type of pH effects we will consider below: specific reactions or set of reactions that are very sensitive to pH and controlled by it in vivo.

There are very few reactions or set of reactions that have been shown to be directly and specifically controlled by pH. Probably the best characterized ones are those reactions which themselves are involved in the regulation of pH_i. It has been shown that the eukaryotic (Aronson, 1985; Grinstein et al., 1985) and the prokaryotic (Bassilana, Damiano & Leblanc, 1984*a*,*b*) Na^+/H^+ antiporters, which have been claimed to be part of the homeostasis mechanism (*see* above) respond to changes in pH_i. Interestingly, both antiporters react in an opposite way, as expected from their proposed roles: the eukaryotic one, which extrudes protons from the cell, is inactive at neutral pH_i and is activated at acid ones; the prokaryotic one, which transports protons into the cell is inactive at acid pH_i. In a fermentative organism in which the H^+-ATPase has been claimed to be part of the regulatory system by extruding protons, the enzyme is inactive above the homeostatic value (Kobayashi, 1985).

Besides control at the level of the activity of the protein, there have been some claims that synthesis of various proteins is regulated by pH. As early as 1943 Gale studied the effect of growing cultures of bacteria at different pH's (Gale, 1943). He found that in cultures grown at alkaline pH, the specific activities of several amino acid deaminases and dehydrogenases were higher than in cultures maintained at neutrality. In cultures grown at low pH, the most dramatic increases were observed in the amino acid decarboxylase. Gale suggested that these phenomena are part of the mechanism of adaptation of *E. coli* to extreme pH's: the resulting amines and carboxylic acids formed tend to bring the pH of the media to neutrality. Nothing is known about the mechanism of this pH effect; is it at the level of enzyme activity and/or synthesis? Is the trigger pH_i and/or pH_o?

In a recent study, Kobayashi, Suzuki and Unemoto (1986) demonstrated that the synthesis of the H^+-ATPase in *S. faecalis* is activated upon acidification of the cytoplasm.

There are most probably other factors playing a

part in pH homeostasis both at the acidic and basic range (*see* V). It is not yet known whether they are triggered by pH_i and/or pH_o.

VII. Regulatory Role of pH_o

A. Bacteria Sense pH_o

Bacteria such as *E. coli* and *Salmonella sp* sense the pH_o by chemoreceptors to protons (Kihara & Macnab, 1981; Repaske & Adler, 1981). It is suggested that the concentration of protons in the extracellular space and, as will be discussed below, in the cytoplasm determine the extent of proton binding to the sensory transducing protein (MCPI). The signal is transduced, by an unknown mechanism, to the motor flagellae, and the bacteria are propelled from the unfavorable pH (Slonczewski et al., 1982).

B. An Alkaline Shift in pH_o Induces the Heat Shock Response in *E. coli*

The heat shock response is a transient response activated upon a temperature upshift (Gottesman, 1984; Neidhardt, van Bogelen & Vaughn, 1984). In *E. coli* a rise of temperature causes an increase in the synthesis of at least 17 proteins. These heat shock proteins (hsps) are under regulation of the *rpoH* (htpr) gene product, a special sigma subunit of RNA polymerase that enables transciption from specific heat shock promoters (Grossman, Erikson & Gross, 1984).

We have recently shown that an alkaline pHo shift (from pH 7 to 8.8) induces the hsps in a manner similar to heat induction with respect to both the kinetics of synthesis and the dependence on the *rpoH* gene (Taglicht et al., 1987). The increment observed was sixfold for *dna*K and *gro*E, fourfold for C62.5 and twofold for F84.1 and sigma70. Hsps were not induced when pH_i was raised by collapsing the ΔpH nor when acid shift of pH_o was conducted. Upon alkaline shifts in pH_o significant induction was observed over a wide range of final pH_o from 6–8.8.

The mechanism by which extracellular pH triggers induction of hsps is not known. Change in pH_o most probably leads to other changes in the cells; for example, an alkaline pH_o shift from pH_0 6 to 8 causes hyperpolarization (Felle et al., 1980; Zilberstein et al., 1979, 1984). We found that heat shock also induces hyperpolarization (*unpublished results* and E.P. Bakker, *personal communication*). However, direct involvement of the change in $\Delta\psi$ in the hsps response has not yet been found. It is also still unknown if the heat shock response is involved in adaptation of bacteria to pH_o.

VIII. Regulatory Role of pH_j

The pH homeostatic mechanism of bacteria is very efficient. Acidification (Kihara & Macnab, 1981) or alkalinization (Zilberstein et al., 1984) of pH_o of *E. coli* perturbs pH_i within seconds. Following either type of change in pH_o, pH_i is restored to the original steady-state value within minutes. Such fast response and precision makes the H^+ concentration of the cytoplasm an attractive candidate to serve as a regulatory signal.

Since the pH_i homeostatic mechanism is an efficient and rapidly responding system, it is technically difficult to study the relationship between change in pH_i and different physiological parameters of wild type bacteria. An experimental approach for this purpose is to induce changes in pH_i and trace their effect to cell processes. As mentioned above, transient failure of pH_i homeostasis in growing *E. coli* cells can be induced by pH_o shifts (Zilberstein et al., 1984), or upon addition of weak acids or bases at a constant pH_o (Kihara & Macnab, 1981).

We have isolated a mutant that fails to regulate pH_i and, therefore, does not grow at alkaline pH_o (higher than pH_o 8.3), although it is fully viable at this pH (Zilberstein et al., 1982). Upon shift back to the permissive pH_o, the mutant recovers and behaves like the wild type. Since at the nonpermissive pH_o the pH_i of the mutant is equal to pH_o, it provides a system to investigate the effect of alkaline pH on different intracellular processes. Similar mutants that possess impaired pH_i homeostatic mechanism at different pH_o will be very helpul for investigation of both the pattern of pH_i regulation as well as regulatory roles of pH_i in the cell.

A. pH_i as a Transducing Signal for Chemotaxis

It has been suggested that pH_i serves as a signal ion for taxis in *E. coli* and *Salmonella* sp. (Kihara & Macnab, 1981; Repaske & Adler, 1981; Slonczewski et al., 1982). Lowering or raising pH_i, by addition of weak organic acids or bases, causes a repellent or attractant response, respectively. Accordingly, changes in pH_o caused transient changes in pH_i, suggesting that the stimulus of a change in pH_o is transduced to the rotor flagellae via a change in pH_i. pH_i has even been suggested to be a converging signal for other repellents and attractants.

Recently it has been shown that addition of ben-

zoate (0.1 M) to *E. coli* increases the internal buffer capacity so that fluctuations in pH_i become unlikely (Eisenbach, Margolin & Ravid, 1985). In spite of this treatment, the chemotactic respone did occur under such conditions. The resulting conclusion that pH_i is not involved in chemotactic signaling was also supported by the observations that attractants or repellents other than weak acids had no effect on pH_i.

B. pH_i and Spore Germination

Using ^{31}P nuclear magnetic resonance, a pH_i of 6 was measured in dormant spores of *B. subtillis* (Barton et al., 1980). Utilizing methylamine distribution, a value of 6.3–6.4 was measured for the dormant spore core region of different Bacilli sp. (Setlow & Setlow, 1980; Swerdlow, Setlow & Setlow, 1981). Upon germination an increase of pH_i to pH 7.3–7.4 was measured. It has been suggested that the low internal pH of the dormant spore contributes to its metabolic dormancy and its resistance. However, the pH_i of *B. megaterium* dormant spores was raised to 7.8 by incubating them with $(NH_4)_2SO_4$ or in the presence of K^+ and there was no detectable effect on the spores ability to germinate or on their dormancy or resistance (Swerdlow et al., 1981). Although spores loaded with H^+ showed decreased resistance properties, it is not clear whether this is due to the lowered pH_i and/or loss of cations. It is possible that a fall in pH_i within developing spores during sporulation plays a role in the acquisition of some spore properties such as dormancy but that subsequently some other changes become the primary mechanism for maintaining dormancy. Alternatively, it is possible that the fall in pH_i may be only an effect of exhaustion of ATP pool during sporulation rather than the cause of this phenomenon.

C. Induction of SOS Functions by Alkaline pH_i

Transient failure of pH_i homeostasis can be caused in *E. coli* by pH_o shifts (Slonczewski et al., 1982; Zilberstein et al., 1984). In a mutant that does not regulate pH_i, the change caused by a shift to an alkaline pH_o is permanent (Zilberstein et al., 1984). Whether transitory or permanent, failure of pH homeostasis was accompanied by cessation of cell division. In the wild type cells, growth resumes 5–10 min after the change in pH_o and in the mutant only upon shift back to the permissive pH_o. In both cases cell division resumed upon recovery of pH_i homeostasis.

Prolonged incubation of the mutant at the nonpermissive pH brought about cell filamentation. Study of the mutant at the nonpermissive pH led to the conclusion that cessation of cell division is not due to a general nonspecific pH sensitivity of the cytoplasm but rather to a specific pH-sensitive function that is related to cell division (Zilberstein et al., 1984).

Inhibition of cell division leading to filamentation is part of the pleiotropic effect of the SOS response to DNA damage (Witkin, 1976; Little & Mount, 1982; Walker, 1984). Therefore, the latter syndrome was investigated upon pH_i perturbation of the mutant and the wild type.

SOS functions are coordinated by two regulatory elements, the *rec*A and *lex*A gene products. The *lex*A protein is a repressor of at least 14 unlinked genes. DNA damage induces a signal which activates a specific protease activity of the *rec*A. Cleavage of the *lex*A repressor by *rec*A protein leads to enhanced expression of the SOS controlled genes, e.g. *uvr*A, *uvr*B, etc., leading to DNA repair, inhibition of cell division and filamentation, etc. (Witkin, 1976; Little & Mount, 1982; Walker, 1984).

We have recently shown (Schuldiner et al., 1986) that raising of pH_i to alkaline values caused an increase in UV resistance in cells of wild type and in the pH sensitive mutant DZ3 of *E. coli*. Utilizing cells transformed with plasmid (pA7) which bears the *uvr*A promoter fused to galK-galactokinase structural gene (Backendorf et al., 1983), it was shown that a shift to alkaline pH_i leads to an increase in the expression of the *uvr*A promoter. An increase of galactokinase activity was monitored in the cells following alkalinization of pH_i. This effect was not displayed in a mutant bearing a *rec*A-insensitive *lex*A gene, nor in cells harboring the plasmid (pAB) in which the galK is fused to an *lex*A-insensitive *uvr*A promoter (Backendorf et al., 1983). Hence, pH_i may trigger the SOS system in *E. coli* cells. The physiological significance of this control relay to pH adaptation is not clear yet. It may provide protection from DNA damage caused by perturbation of pH_i. It may also reflect other, more general, role of pH_i.

D. pH_i in Eukaryotic Systems

Eukaryotic systems are beyond the scope of this review and the reader seeking for detailed information is referred to some recent publications that summarize most of the findings (Nuccitelli & Deamer, 1982; Grinstein et al., 1985*a*,*b*; Pouyssegur, 1985; Moolenaar, 1986). However, for the sake

of completeness we will mention some of the most salient findings.

In this field, as in many others, the development of probes tailor-made for the measurement of pH_i has resulted in an explosion of data in which changes in pH have correlated with changes in cell growth and metabolism. Virtually all growth-promoting agents stimulate an amiloride-sensitive, Na^+/H^+ antiporter in a variety of quiescent cells. As a result of this activation, the intracellular pH increases by 0.15–0.3 pH units (Schuldiner & Rozengurt, 1982; Moolenaar et al., 1983; L'Allemain, Paris & Pouyssegur, 1984; Grinstein et al., 1985*a*,*b*; Pouysseur, 1985; Moolenaar, 1986). This activation is due to a shift in the dependence of the antiporter on pH_i. A clue to the possible mechanism of such a regulatory phenomenon can be found in recent results in which activators of protein kinase C have been shown to affect the antiporter in an identical way (Nishizuka,1984). In addition to the above findings, we should mention that fertilization of a variety of ova (Johnson, Epel & Paul, 1976) and germination of yeasts (Barton et al., 1980) and bacterial spores (*see above* section VIIIB) also show a characteristic increase in pH_i which precedes the cascade of changes in cell metabolism.

IX. Other systems in which Regulatory Role of $\Delta\tilde{\mu}_{H^+}$ Have Been Suggested

We will mention here systems in which the energetic or regulatory role of the $\Delta\tilde{\mu}_{H^+}$ (or its components) have not yet been clarified. Requirement for $\Delta\tilde{\mu}_{H^+}$ as an energy source has been suggested for protein translocation across the cytoplasmic membranes (Daniels et al., 1981; Zimmerman & Wickner, 1983; Bakker & Randall, 1984; Rhoads, Tai & Davis, 1984; Chen & Tai, 1985). Recently, however, it has been shown that the driving force for translocation of alkaline phosphatase and *ompA* protein is most probably ATP. The $\Delta\tilde{\mu}_{H^+}$ may only affect the efficiency of the process by either an energetic and/or regulatory role (Chen & Tai, 1985).

An effect of membrane potential has been demonstrated on nitrogen fixation by bacteroids of *Rhizobium leguminosarum* (Laane et al., 1979). The effect has been related to an effect of $\Delta\psi$ on the formation of reducing equivalents in this bacterium.

An effect of membrane potential has also been shown on enzyme II of the phosphotransferase system (Haguenauer & Kepes, 1972; Klein & Boyer, 1972; Postma & Lengeler, 1985). Studying this system in inverted membrane vesicles, it was suggested that the $\Delta\tilde{\mu}_{H^+}$ inhibits the enzyme by raising its K_m for methyl-α-D-glucoside (Robillard & Konings, 1981, 1982). The implications of these data are still debatable (Postma & Lengeler, 1985).

X. Concluding Remarks

The data surveyed in this review suggest that $\Delta\psi$, pH_o and pH_i may have regulatory roles in the bacterial cell. This conclusion is based almost solely on the correlations of in vivo systems data. Therefore, until higher resolution of each of the systems is achieved, it will be very difficult to prove a direct control effect.

A requirement for $\Delta\psi$ was implicated for cellulose synthesis in *Acetobacter xylinum*, for processes involving DNA penetration into cells including plasmid transformation, conjugation and phage DNA injection. Phage adsorption which precedes the injection causes depolarization of $\Delta\psi$. The mechanism of modulation by $\Delta\psi$ is not known in any of the processes, however, $\Delta\psi$ across phospholipid vesicles results in a substantial change in fluidity of the bilayer (Lelkes, 1979), its thickness and probably exposure of proteins (Shinitzky et al., 1983). The $\Delta\psi$ may also affect the polarity of distribution of the lipids or the proteins and, therefore, influence the availability of specific sites and/or their conformation. For mechanistic consideration, it is of interest that a similar threshold of a $\Delta\psi$ of 60–80 mV was found both for cellulose synthesis (Delmer et al., 1982) and T4 DNA injection (Labedan et al., 1980). Such findings suggest that these phenomena respond to some as yet unidentified trigger mechanism.

The sites of regulation by pH are still unknown and remain to be established. At least two hypothetical levels of action can be suggested at present: (i) pH affects the rate of synthesis of a specific protein or sets of proteins. Thus, synthesis of the H^+/ATPase has been shown to be influenced by pH (Gale, 1943; Kobayashi, 1986).

(ii) pH affects the structure and/or activity of specific existing proteins. Thus effects on the Na^+/H^+ antiporter or the H^+-ATPase have been suggested to play role in pH_i regulation.

The mechanism by which pH_o and pH_i induce the repellent signal is obscure; however, a protein with an H^+ binding site has been suggested (Slonczewski et al., 1982).

In the case of the activation of the heat shock response, there may be a receptor protein in the membrane or in the periplasmic space that transduces changes in pH_o in a still unknown way.

In the case of effect of pH_i on the SOS system, it can be that the effect is mediated secondarily by changes such as damage to the DNA caused by

alkaline pH_i and not to direct triggering of the SOS response. However, it can also be more direct via a highly pH-sensitive protein. A very good candidate for such a protein is the *lex*A product itself. It has been shown in vitro that this protein exhibits autodigestion activity upon shift to alkaline pH (Little, 1984). Whether this phenomenon is the basis of the SOS induction by alkaline pH_i is not yet known.

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