The Sodium/Proton Antiporter Is Part of the pH Homeostasis Mechanism in Escherichia coli*

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The Escherichia coli chromosome has been shown to bear at 89.5 min a locus designated pha which determines the Na⁺/H⁺ antiporter activity. The mutant DZ3 has previously been shown to be simultaneously impaired in Na⁺ extrusion capacity and in growth on the Na⁺-co-transported substrates, melibiose and glutamate (Zilberstein, D., Padan, E., and Schuldiner, S. (1980) FEBS Lett. 116, 177–180). This mutant when mated with the wild type yielded wild type-like recombinants that appeared at a map distance of 1.5 min from metB recombinants. Furthermore, genotypes containing repressed operon of glutamate which cannot grow on this substrate still bear the normal pha locus and served as donors for transduction of DZ3 to yield wild type-like transductants.

The mutant DZ3 also has been shown (Zilberstein et al., see above) to be impaired in growth at alkaline pH. This fact allowed us to investigate the role of the Na⁺/H⁺ antiporter in pH homeostasis in E. coli. A pH-controlled growth system and rapid filtration technique were used to compare the wild type and the mutant DZ3 with respect to both internal pH and growth during transfers of logarithmically growing cells to different external pHs. Following the shift in external pH of the wild type, a transient state was initiated by reduction of ΔpH across the membrane. Subsequently, at a specific time course pH homeostasis was re-established. Whereas the capacity of the pH homeostasis mechanism was found to be a function of both the span of the external pH shift as well as the rate at which the change occurs, inhibition of protein synthesis did not affect this process. After stepwise transfer of growing wild type cells from pH 7.2 to 8.3 to 8.6, and then to 8.8, or from 7.2 to 6.4, the ΔpH was initially zero at each step and growth ceased. Subsequently, within 6 min at the most, the ΔpH was built up to a magnitude that yielded an internal pH of 7.6–7.8 and thereafter growth was resumed at the initial rate. However, if the shift was made abruptly from pH 7.2 to 8.6, the lag was longer and the buildup of the ΔpH was slower. The shift between pH 7 to 8.8 appeared to be the limit of the pH homeostasis capacity since the wild type grew normally when allowed to adapt by step transfers over this range and failed to restore both normal internal pH and optimal growth if the transition was made in one step.

Since the mutant DZ3 behaved like the wild type after transfer from pH 7.2 to 6.4, but exhibited progressive failure to control internal pH and to grow at the alkaline shifts, we conclude that the Na⁺/H⁺ antiporter is an absolute requirement for pH adaptability at alkaline pH. It is suggested that the collaborative functioning of this antiporter with the primary proton pumps extruding protons is the basis of the pH homeostasis mechanism at alkaline pH.

In all cases, both in the wild type and the mutant, recovery of pH homeostasis always preceded initiation of growth, indicating a tight coupling between the two processes.

Most proteins, even those isolated from alkalophilic and acidophilic bacteria, have a narrow pH range of optimum activity and/or stability which falls at around neutrality (1). It is therefore not surprising that a mechanism maintaining the cytoplasmic pH constant at around pH 7.6 was found to form the basis of adaptability to pH in many bacteria (reviewed in Refs. 2 and 3). Furthermore, because in the prokaryotic cell, unlike the eukaryotic cell, the primary proton pumps are located at the cytoplasmic membrane in direct contract with the medium, these pumps have been suggested to be involved in the pH homeostasis mechanism (4, 5) in addition to their established role in energy conversion (6, 7). Thus, when the cell proton pumps linked to electron transport (4), ATP hydrolysis (5), or photochemical reaction (8) are inhibited, the protons equilibrate across the membrane. Upon resumption of the activity of these pumps, a ΔpH is built to a magnitude dependent on the pHₐ (9) so that a constant pHₐ of 7.6 is maintained over a wide range of pHₑ. Like the intact cell, membrane vesicles isolated from bacteria have the propensity to maintain a constant internal pH during changes in pHₑ (9, 10). This physiological facet of the primary proton pumps appears to be a general phenomenon of the prokaryotic cell and many bacteria, both neutrophiles like Escherichia coli as well as acidophiles and alkalophiles, exhibit pH homeostasis in a similar pattern: the proton pumps maintain a ΔpH sensitive to pHₑ (review in Refs. 2 and 3).

It is evident that the mechanism by which the ΔpH across the cytoplasmic membrane of the prokaryotic cell varies with pHₑ forms an essential part of the pH homeostasis of bacteria. It has been suggested that the Na⁺/H⁺ antiporter is involved in this mechanism in E. coli (4). Evidence has been presented supporting the presence of the Na⁺/H⁺ antiporter in many energy transducing membranes (11), including that of E. coli (12–14). Energy-dependent extrusion of Na⁺ from E. coli cells has long been observed (15) and has been shown to be dependent on the proton gradient maintained across the membrane both in intact cells (12, 17) and in right-side-out vesicles.

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† The abbreviations used are: pHₑ, external pH; pHₐ, internal pH; DMO, 5,5-dimethyl-2,4-oxalasiddenedione; pH*, phenotype of growth sensitivity to medium pH.
Na⁺/H⁺ Antiporter and pH Homeostasis in Escherichia coli

In an anoxic cell suspension of E. coli, after H⁺ ions have been translocated outward across the cytoplasmic membrane by a respiratory pulse (12) or inward by a lactose pulse (16), re-equilibration is catalyzed by the presence of Na⁺. Similarly Na⁺ fluxes reduce the ΔpH (acidic side) across right-side-out membrane vesicles (13). Furthermore, in line with its suggested role in the control of the ΔpH with pHₐ, the Na⁺/H⁺ antiporter has been shown to be pH-dependent. The system was found to be electrogenic with a higher rate at high pH than at acidic pH where it is electroneutral (13, 16).

Further analysis of the function of the Na⁺/H⁺ antiporter of E. coli has recently become possible by the isolation of a mutant (DZ3) which is impaired in all the Na⁺/H⁺ antiporter-related activities (17, 18). This antiporter is the only system known in E. coli which extrudes Na⁺ and maintains a sodium gradient that is directed inward and acts as a driving force for the uptake of substrates that are co-transported with Na⁺. The mutant, therefore, was selected by its inability to grow on glutamate and melibiose, two substrates that are co-transported to the cell with Na⁺ (19–21). Accordingly, the mutant was found to be defective in its sodium extrusion capacity. In the present study, we have extended our preliminary genetic data (17) to show that, indeed, a single locus designated phs and mapped at 89.5 min is mutated in DZ3 and, therefore, it most probably determines the Na⁺/H⁺ antiporter activity in E. coli.

Strikingly, the mutant DZ3 lost the capacity to grow at alkaline pH, but grew like the wild type up to pH 7.5 (17). This pH sensitivity of growth of DZ3 may be caused by an effect of the phs mutation on control of pH. Evidently, in this case, this mutant may serve as a powerful experimental tool to demonstrate the role of the Na⁺/H⁺ antiporter in the pH homeostasis mechanism of E. coli. To test this possibility, the wild type and the mutant were compared with respect to adaptability to changes in pH. To exclude a very recent paper E. coli studies were designed for determination of the final steady state value of pHi maintained by the cells and no attention was paid to the transient stages following shift in pH, (review in Refs. 2, 3). For the study of the pH homeostasis mechanism and its relation to growth in both the wild type and the mutant, we therefore used a pH-controlled growth system and a rapid filtration technique for cell separation. This experimental procedure allowed us to monitor rapidly and continuously changes in growth and in pH, induced in growing cells by changes in pHₐ, of the culture and hence unraveled the details of the pH homeostasis mechanism of growing cells. It has been found that following a change in pHₐ, of growing cells the ΔpH immediately diminishes across the membrane, and only then, at a specific time course and with no need of protein synthesis, the pH homeostasis is re-established; the capacity and the time course of the pH homeostasis mechanism is a function of both the span of the change in pHₐ and the rate at which the change occurs; pH homeostasis is a prerequisite for growth and finally and most importantly the Na⁺/H⁺ antiporter plays a primary role in the pH homeostasis mechanism of E. coli at alkaline pH.

**EXPERIMENTAL PROCEDURES**

**Bacteria and Growth Media**—The E. coli K12 strains used are described in Table I. Cells were grown on minimal medium A (22) lacking citrate, supplemented with L-methionine (50 μg/ml) and containing 0.5% glycerol as the carbon source. Solid medium was prepared by the addition of 1.5% Difco agar. L broth used for transduction and conjugation contained KCl instead of NaCl.

**Transduction**—P1 lysates of the donor bacteria were prepared and transduction was done as previously described (23, 24).

**Mating Experiments**—Mating experiments were performed as described elsewhere (25).

**Growth under Controlled pH**—Cells were inoculated into the growth medium in which the MgSO₄ concentration was reduced to 0.001%. To control the pH of the growth medium, cells were grown in a BioFlo Model C30 chemostat (New Brunswick Scientific) as batch cultures at 37 °C. The pH of the medium was controlled by means of a Modcon (Kryat Motzkin, Israel) pH titrator. KOH or HCl were added at a rate of 2.25 or 3.6 meq/min, respectively.

**Determination of Intracellular pH under Growth Conditions**—Intracellular pH was evaluated during growth from the distribution across the cell membrane of either [14C]DMO or [14C]methylamine (26). Since the aim of the work was to measure pHₐ at the logarithmic growth phase at different and well defined external pH, it was essential to reduce experimental manipulation to the minimum to avoid distortions in the measurements. Ten ml of a cell suspension (0.1–0.17 mg of cell protein/ml) were immediately transferred from the chemostat into a prewarmed (37 °C) 100-ml flask containing 0.8 μM [14C]methylamine (68 Ci/mol) or 0.32 μM [14C]DMO (120 Ci/mol). The suspension was incubated for 1 min with continuous shaking at 37 °C and filtered through a glass fiber filter (GF/C Whatman, 25-mm diameter). The use of these filters allowed the use of large amounts of cell protein which increased the sensitivity of the measurement, and washing could be avoided. The filters were transferred into toluene/Triton scintillation liquid and assayed for radioactivity in a Tricarb scintillation counter. The amount of radioactivity retained on the filters in the absence of ΔpH, namely at pHₐ 7.6 (4) or at pH 7 in the presence of carbonyl cyanide p-trifluoromethoxyphenyl hydrazone, was identical and served as the blank. The steady state level of uptake of the ΔpH probe at all pH values was attained within 2 s. To calculate the concentration ratio, the volume of the filtered cells was estimated from the amount of cell protein retained on the filters using the factor 5 μl of csmotic water/mg of cell protein (4).

**Protein Determination**—Protein was determined by the Lowry method (27). [14C]Proline, [14C]DMO, and [14C]methylamine were from New England Nuclear.

**RESULTS**

The mutant DZ3 has been shown to be simultaneously defective in growth on glutamate and melibiose and at alkaline pH and in its energy-dependent sodium extrusion capacity (17). It was therefore crucial to demonstrate that a single locus is involved in the mutation of strain DZ3. Table II

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**Table II**

| Conjugants          | Recombinants | Linkage | Mapping point |
|---------------------|--------------|---------|---------------|
| Strains             | Phenotypes   | Selected phenotypes | Unselected phenotypes | % | min |
| Donor, CS72         | Wild type    | Mel⁺ | Glt⁺ | 100 | 89.5 |
| Recipient, DZ3      | Glt⁺, Mel⁺, pH⁺ | Mel⁺ | Mel⁺ | 48 |         |
|                     | Glt⁺ | Mel⁺ | 100 | 89.5 |
|                     | Glt⁺ | Mel⁺ | 100 | 88 |
|                     | Met⁺ | Glt⁺ | 100 | 100 |

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**Table I**

| E. coli K12 strains used in this study |
|---------------------------------------|
| Strain | Mating type | Genotype | Source/reference |
|--------|-------------|----------|------------------|
| CS71   | Hfr         | gltC metB lacY1 | (17) |
| CS72   | Hfr         | gltC lacY1 | (17) |
| DZ3    | Hfr         | gltC metB lacY1 | (17) |
| DZ31   | Hfr         | gltC metB lacY1 | (17) |
|        |             | phs      |                  |
| CS101B | Hfr         | gabC     | Y. S. Halpern (23) |
| M2719  | F'          | mel7 lacY galK str' | R. Schmidt, 1968 (38) |
summarizes the results of conjugation experiments between the mutant to which nalA was transduced (DZ31), and a derivative of the wild type (CS72). In these strains, metB maps exactly at half the distance between the mel and glt loci and mel is expected to penetrate first (20). Recombinants capable of growth on melibiose were obtained and all were able to grow on glutamate, suggesting that a single locus (phs) is responsible for growth on both carbon sources. Accordingly, all the Glt+ recombinants were Mel+. The Mel+ recombinants tested in the presence of methionine appeared all the Glt' recombinants were Mel'. The Mel+ recombinants is responsible for growth on both carbon sources. Accordingly, followed. For example, upon transfer of wild type cells from

The mutation of DZ3 maps in a locus at 89.5 min on the E. coli chromosome which we designate phs (Table II). This locus is far from the operons responsible for the utilization of both glutamate (81.7 min) and melibiose (92.5 min) (39). Hence, genotypes of the latter two operons, which determine phenotypes that cannot grow on the respective carbon sources (Glt- or Met-) must have a normal phs allele and should therefore still serve as donors for transduction of DZ3 to yield wild type transductants. Such a transduction experiment is described in Table III. Whether selected for Mel+ or for Glt+, the transductants were simultaneously both Glt+ and Met+ and grew at high pH, suggesting that transduction of the phs wild type allele had occurred, thus curing the pleiotropic effects of phs. The Lac- phenotype determined by the y locus, which is well separated from phs, was not co-transduced with either Glt+ or Mel+.

It is concluded that the locus phs determines the sodium proton antiporter as it is the common denominator for the functions affected pleiotropically in DZ3.

It has been proposed that the Na+/H+ antiporter has a role in regulation of internal pH (4, 12). Hence, the finding that DZ3 does not grow at alkaline pH can be attributed to its lack of pH homeostasis at the alkaline pH. Internal pH has been measured both in the cells of the wild type and the mutant following transfer between different pH values. Cells were grown in a pH-stat at pH 7.2 to the logarithmic phase and a ΔpH of 0.55 units (alkaline inside pHi = 7.75) was found. Either KOH or HCl were then automatically added to yield a new preset pH and both internal pH and growth were followed. For example, upon transfer of wild type cells from pH 7.2 to 8.3, the ΔpH dissipated immediately and growth ceased (Fig. 1A). Within a lag of about 10 min, the ΔpH of 0.5 was built up yielding an internal pH of 7.8 and growth subse-

![Fig. 1. Growth and ΔpH after shift of external pH from 7.2 to 8.3. CS71 (A) and DZ3 (B) cells were grown in a pH-stat at pH 7.2 to midlogarithmic phase (0.1 mg of cell protein/ml). KOH was automatically added and the final pH 8.3 was reached after 3 min. At given times, samples were withdrawn for determination of growth (•) and ΔpH (○).](http://www.jbc.org/)

![Fig. 2. Doubling time and pH, following stepwise changes in pH. CS71 (A) and DZ3 (B) cells were grown in a pH-stat at pH 7.2 to midlogarithmic phase (0.1 mg of cell protein/ml). KOH was automatically added to yield pH 8.3 as described in Fig. 1. After about one generation, the cells were diluted 3-fold with fresh buffer at the same pH and temperature. After growth to 0.1 mg of cell protein/ml, the pH was shifted to 8.6 by the addition of KOH. In the same manner as described above, the cells were further shifted to pH 8.8. Similarly, shift to pH 6.0 was achieved by addition of HCl to cells previously grown at pH 7.2. At each step, both growth (•) and ΔpH (○) were determined.](http://www.jbc.org/)

| TABLE III |
|----------|
| Transduction of phs gene into DZ3 | |
| Transduction of DZ3 was done using P1 phage (24). | |

| Donor strains | Phenotype | Classes of DZ3 transductant | No. of transductants | Frequency of co-transduction |
|--------------|-----------|-----------------------------|----------------------|-----------------------------|
|              |           | Lac- | Mel+ | Glt+ | % |
| CS71 Wild type | Mel+ | 130  | 0    | 100  | |
| CS101B Glt+ | Glt+ | 112  | 0    | 100  | |
|             | Mel+ | 150  | 0    | 100  | |
|             | Lac+ | 150  | 0    | 100  | |
| M2719 Mel+ | Mel+ | 100  | 0    | 100  | |
|             | Glt+ | 110  | 0    | 100  | |

It is evident that following the shift in pH, a perturbation of pH was observed and the ΔpH immediately diminished across the membrane (Figs. 1, 3, and 4). The rise in ΔpH started only after a lag period of at least 3 min. To test whether this lag was due to a requirement for the synthesis of a new protein, chloramphenicol was introduced to the growing culture 3 min before the pH shift was done. It is shown in Fig. 4 that whereas both protein synthesis and growth were arrested in the presence of the inhibitor, the ΔpH was normally rebuilt and maintained for at least 45 min.

As compared with the wild type, during the shift between pH 7.2 and 8.3, the mutant DZ3 showed a longer lag of 20 min before the ΔpH was built up and the ΔpH established was smaller (Fig. 1F). Accordingly, growth of the mutant resumed only after 20 min and at a slower rate (100-min doubling time) than that of the control (Fig. 1B). Further stepwise transfer of the mutant to pH 8.6 and then to pH 8.8 progressively slowed now the growth to complete cessation. Remarkably, the pH homeostasis failed with increasing pH and at pH 8.6.
and higher, pH was almost equal to the external pH. It is of interest that growth and pH were normal both at pH 7.2 and 6.5 (Fig. 2B).

When the transfer from pH 7.2 was made directly to pH 8.6 (Fig. 3) without an “adaptation” period at pH 8.3, DZ3 completely failed to resume growth even during a period of 18 h. The pH homeostasis initially failed even in the wild type for the first minutes after such a pH jump. However, ΔpH was eventually built up at 20 min and shortly thereafter was resumed (Fig. 3A). This pH span may indeed be the limit of the homeostasis machinery capacity since after a wider span (7.2 → 8.8) the normal pH was not recovered and growth resumed at a very slow rate (Fig. 3B). A still larger shift (7.2 → 8.8) cannot be coped with at all, even by the wild type. It is clearly evident that, at alkaline pH range which does not permit its growth (17), the mutant DZ3 is impaired in pH homeostasis.

We may conclude, therefore, that the Na+/H+ antiporter which is the primary site of lesion in this mutant is required for pH homeostasis at alkaline pH, and that the pH homeostasis is a prerequisite for optimal growth. Indeed, tight coupling was observed between normal pH and growth, both in the mutant and the wild type.

Mutant cells maintained at the nonpermissive pH of 8.6 for at least 8 h were fully viable and when transferred back to pH 7.2 they restored pH, to 7.6–7.8 and grew at the normal rate (not shown). It is therefore highly suggestive that it is the lack of pH homeostasis which hinders growth of DZ3 at alkaline pH. Accordingly, optimal growth of the wild type was observed when pH homeostasis was restored. The wild type maintained constant pH, of 7.6–7.8 and grew optimally up to pH 8.8 (Fig. 2A). At pH 8.8, it grew normally if allowed to adapt slowly by step transfers but failed to restore both ΔpH (final pH, was 7.9) and normal growth if the transition was made in one step (compare Fig. 2 to Fig. 3). At pH 9, there was no ΔpH, growth ceased, and viability was reduced to 50% within 6 h. Both in the wild type and the mutant, recovery of pH always preceded initiation of growth.

**DISCUSSION**

This study describes the use of a pH-controlled growth system and rapid filtration technique to investigate the pH homeostasis mechanism in growing *E. coli* cells. The bacteria were analyzed after exposure to different pH stresses and our previous results with resting cells (4) were extended. Following a shift in pH, a transient stage of perturbed pH was clearly observed before pH homeostasis was restored. Thus, upon stepwise transfer of growing wild type cells from pH 7.2 to 8.3 then to 8.6 and 8.8, or from pH 7.2 to 6.4, the ΔpH was found to be zero initially at each step. Subsequently after a lag of several minutes, the ΔpH reached a magnitude that yielded a constant internal pH of 7.6–7.8 (Figs. 1A and 2A).

Most probably during this transient period, the pH homeostasis mechanism is readjusted to the new state. It is remarkable that this process was found not to involve the synthesis of a new protein (Fig. 4). Since equilibration of the protons across the membrane initiated the transient stage, it is suggested that pH, pH, or both may serve as the signal for the new adjustment of the pH homeostasis.

The capacity of the pH homeostasis mechanism was found to be a function of both the span of the shift in pH, and the rate at which the change occurred. A change in pH from 7.2 to 8.6 was followed by a longer lag for the ΔpH (20 min) than the sum of all the lags observed (about 12 min) during stepwise shifts of external pH to the same final value (7 → 8.3 → 8.6) (Fig. 2A). One possible explanation for these differences in the duration of the lag is very likely a greater leak of intracellular material that occurred after the more drastic shift, and which could impede the rate of restoration of the ΔpH. The shift between pH 7 to 8.8 appeared to be the limit of the capacity of the pH homeostasis mechanism in the alkaline range since pH, is not restored to the normal value following such a stress.

The conjugation and transduction experiments conclusively showed that a single locus, *phs*, maps at 89.5 min on the *E. coli* chromosome, determines the Na+/H+ antiporter, and is mutated in DZ3. As this mutant was also found to be incapable of growth at alkaline pH (17), it was used here to study the role of the Na+/H+ antiporter in the pH homeostasis of *E. coli*. As compared with the wild type, mutant DZ3 behaved normally after the pH jump from 7.2 to 6.4. At the first alkaline transfer to pH 8.3, however, there was a marked increase in the lag up to 20 min. The internal pH reached was only 8 and the growth was resumed at a rate twice as slow as that in the wild type. At further increase in pH, the pH homeostasis progressively failed and beyond pH 8.6 the protons equilibrated across the membrane and growth ceased. Hence, it was concluded that the Na+/H+ antiporter is indeed needed for pH homeostasis at alkaline pH.

In view of these results, the pH homeostasis mechanism of *E. coli* can now be reanalyzed. It was previously shown that the pH homeostasis system is based on the primary proton pumps. These maintain the ΔpH which changes with external pH so that the internal pH is kept constant at pH, 7.6–7.8 (4).
Thus, below pH 7.5, the pH is alkaline inside, and above this pH, acidic inside, suggesting two patterns of control of the ΔpH, above and below pH 7.6-7.8. Indeed, the functionality of the Na+/H+ antiporter, affected by the mutation of DZ3, is not required at the acidic pH. The outward directionality of the primary proton pumps is consonant with the inward alkaline orientation of the ΔpH observed up to pH 7.6. The rate of respiration remained unaltered all over this external pH range, suggesting a constant rate of proton pumping (4) but does not explain the drastic decrease in ΔpH observed. However, the observed increase in ΔΨ with increasing pH (16) which is also maintained by these pumps is in accordance with the decrease in the ΔpH, since, as in many other systems, here too, ΔΨ limits the number of protons that can be pumped out (4). This change in ΔΨ with pH implies that electrogenic movement of ion(s) is pH-dependent in E. coli and forms part of the pH homeostasis as previously suggested for Vibrio alginolyticus (30).

Above pH 7.6-7.8, it is clear that an increase in ΔΨ does not account for the changes in the ΔpH occurring in the alkaiic range: the ΔΨ hardly changes and is still negative inside when the ΔpH reverse and becomes acidic inside (16). In the present study, we clearly show that the mechanism for the reversion of ΔpH above pH 7.6 involves the Na+/H+ antiporter. The Na+/H+ antiporter must recycle the protons extruded by the primary pumps to the cell. The antiporter has previously been shown to be electroneutral at alkaline pH (13, 40) as well as pH-sensitive (16). It is envisaged that the rate of translocation of protons by the antiporter, relative to the protons pumped out, is such as to yield an overall net influx of protons required for the ΔpH, while positive charges are still directed outward (2, 3, 13). Hence, it is concluded that the cooperative action of the proton pumps and the Na+/H+ antiporter constitutes the pH homeostasis mechanism at the alkaline pH range.

Although a sodium requirement for growth has not been shown in E. coli, it should be emphasized that almost every commercially available compound as well as glassware is contaminated with Na+ and can yield up to 0.1 mM Na+ (31). For this reason, the possibility that bacteria require a low sodium concentration has not yet been ruled out. Nevertheless, the participation of more than one antiporter system in regulating internal pH is not mutually exclusive and the relative contribution of each may change under different environmental conditions. Indeed, a K+/H+ antiporter has recently been suggested to play a role in pH homeostasis in E. coli (32–34).

The capacity for maintaining a constant cytoplasmic pH is not unique to E. coli. Many other bacteria neutrophiles as well as acidophiles and alkalophiles maintain a constant pH, (reviewed in Refs. 2 and 3). Furthermore, the mechanisms of pH homeostasis seem common to all with proton pumps playing the primary role. In the case of the alkalophiles similar to E. coli, the Na+/H+ antiporter has been conclusively shown to take part in the pH homeostasis mechanism at alkaline pH (35, 36).

Given the pH sensitivity of most enzymatic reactions and the high efficiency of the pH homeostasis mechanism in bacteria, it is suggested that changes in pH may serve as regulatory signals of cell physiology. Indeed, in the present study, we observed a tight coupling between pH and growth. Thus, in all cases both in the wild type and the mutant DZ3, recovery of pH, always preceded initiation of growth. Furthermore, this mutant which was found to be impaired in pH homeostasis at alkaline pH remained viable for many hours at the nonpermissive pH yet it did not grow. Only when pH homeostasis was restored at lower pH was growth resumed. Other recent observations indicate the possible physiological role of changes in pH. It has been shown that, in two Bacillus species, the pH within the dormant spore is around 6.3 and it rises to 7.5 upon germination (37). Reversible perturbation of pH has been shown to be involved in pH taxis in E. coli (29).

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