JOURNAL OF BACTERIOLOGY, Nov. 1986, p. 936–939
0021-9193/86/110936-04$02.00/0

Vol. 168, No. 2

Induction of SOS Functions by Alkaline Intracellular pH in *Escherichia coli*

SHIMON SCHULDINER,[1]* VERED AGMON,[2] JOURICA BRANDSMA,[3] AMIKAM COHEN,[4] EINAT FRIEDMAN,[2] AND ETANA PADAN[2]

Departments of Molecular Biology,[1] Molecular Ecology,[2] and Molecular Genetics,[4] Institute of Microbiology and Institute of Life Sciences, Hebrew University, Jerusalem, Israel, and Department of Biochemistry, State University of Leiden, Leiden, The Netherlands[3]

Received 21 October 1985/Accepted 7 August 1986

Alkalinization of intracellular pH (pH_i) causes an increase in UV resistance in wild-type and pH-sensitive mutant (DZ3) cells of *Escherichia coli*. Utilizing cells transformed with a plasmid (pA7) which bears the *uvrA* promoter fused to *galK* galactokinase structural gene, it was shown that alkaline pH_i leads to an increase in the specific activity of galactokinase. This effect was not displayed in a mutant bearing a *recA*-insensitive *lexA* gene, nor in cells harboring a plasmid (pA8) in which the *galK* is fused to a *lexA*-insensitive *uvrA* promoter. Hence, the effects of pH_i on cells functions may involve the *lexA* product of the SOS system.

Growing bacteria actively regulate their intracellular pH (pH_i) over a wide range of extracellular pH (pH_o) values (15, 16). Redox- and ATP-driven proton pumps are involved in most cases studied until now. Coupled to the primary extrusion of protons, other electrogenic unidirectional and exchange ion systems seem to play a role in pH_i homeostasis (2, 9, 15, 16, 21).

Transient failure of pH_i homeostasis in growing *Escherichia coli* cells has been detected upon shifts of pH_o (21, 22) or upon addition of weak acids or bases at a constant pH_o (8). In addition, we have described a mutant that fails to regulate pH_i at alkaline pH_o values (higher than 8.3) (21–23). Whether transitory or permanent (in the mutant at alkaline pH), failure of pH homeostasis is accompanied by cessation of cell division. In the wild-type cells, growth resumes 5 to 10 min after the change in pH_o, and in the mutant, growth resumes upon shift back to the permissive pH. In all cases, cell division resumes upon recovery of pH_i homeostasis. Prolonged incubation of the mutant at the nonpermissive pH brings about filamentation (22).

It was previously suggested that cessation of cell division upon failure of pH homeostasis is not due to a general nonspecific pH sensitivity of cytoplasmic proteins. Thus, the energy metabolism of the cell as measured by respiration, the size of the proton electrochemical gradient, and the rate of solute transport was not significantly affected in the mutant even 2 h after transfer to the nonpermissive pH_o. In addition, the induction ability of β-galactosidase, which requires both transcription and translation, was constant for at least 40 min after the shift (22). It seems, therefore, that there is a specific pH-sensitive function that controls cell division.

Inhibition of cell division leading to filamentation is part of the pleiotropic effect of the SOS response to DNA damage (12, 19, 20). SOS functions are coordinated by two regulatory elements, the *recA* and *lexA* gene products. The *lexA* protein is a repressor of at least 14 unlinked genes. DNA damage induces a signal which activates a specific protease activity of the *recA* protein. Cleavage of the *lexA* repressor by the *recA* protein leads to enhanced expression of the SOS-controlled genes, e.g., *uvrA*, *uvrB*, etc. One of the first reactions upon SOS induction is the inhibition of cell division, which is controlled by the SOS-inducible *sfiA* gene.

On the basis of the properties of pH_i homeostasis and cessation of cell division in both the wild type and the mutant, we tested a possible relation between alkalinization of pH_i and induction of the SOS system. In this communication we present evidence that alkalinization of pH_i in *E. coli* brings about the induction of SOS-related functions.

MATERIALS AND METHODS

Bacterial strains and plasmids. The bacterial strains used in this study are listed in Table 1. The two plasmids used, pA7 and pA8, are fusions of the *uvrA* promoter with the structural *galK* gene (1). Plasmid pA7 contains the complete *uvrA* promoter and operator (SOS box) region, whereas in pA8 the SOS box and therefore also the −35 sequence of *uvrA* have been deleted. Nevertheless, pA8 shows a basal noninducible level of *galK* transcription (1).

Growth under controlled pH. Cells were grown on minimal medium (4) lacking citrate, with 1/10th the $MgSO_4$ concentration and supplemented with the appropriate amino acids (50 μg/ml) and thiamine (1 μg/ml). Ampicillin (50 μg/ml) was added to plasmid-containing strains. The carbon source was glycerol (0.5%). pH was controlled by means of a Modcon (Kiryat Motzkin, Israel) pH titrator. KOH or HCl was used to change the pH. These were added at rates that give changes of 0.2 to 0.3 pH units/min.

Effect of UV irradiation on cell survival. At appropriate time periods cells were serially diluted in ice-cold medium A lacking glycerol and magnesium. Various dilutions were spot plated on LB solid medium. Parallel samples were irradiated by UV light (Minerlight UVS-12) for various time intervals. Plates containing irradiated bacteria were incubated at 37°C in the dark for 10 to 12 h. The time required for 90% killing (T_{90}) was determined from individual killing curves for each time point.

Enzyme assays. For the assay of β-lactamase and galactokinase activity, 1-ml cell suspensions were collected by centrifugation. The pellets were kept at −20°C until assay. Galactokinase (1) and β-lactamase (3) activities were assayed as previously described.

* Corresponding author.

TABLE 1. Strains used

Strain designation	Relevant genotype	Source or reference
CS71	*gltR metB lacYI*	23
DZ3	*gltR metB lacYI phs*	23
C600-*glt*		This laboratory
AB1157	*ssb*$^+$	6
JGC206	*ssb-1 zjb-1*::Tn*10*	5
CS4804	*ssb*$^+$ *zjb-1*::Tn*10*	P1(JGC206) AB1157; selection for Tet^r and UV^r
DM49	*lexA3*	13
CS4808	*lexA3 zjb*::Tn*10*	P1(CS4804) → DM49; selection for Tet^r and UV^s
CS71 *lexA3*	As CS71, but *lexA3*	P1(CS4808) → CS71; selection for Tet^r and UV^s
T100	*thy his srl*::Tn*10*	Derivative of Hfr G6
CS71 *recA*	*recA srl*::Tn*10*	P1(T100)CS71; selection for Tet^r and UV^s

Determination of pH_i. pH_i was determined from the distribution across the membrane of [^{14}C]5,5-dimethyloxazolidine-2,4-dione or [^{14}C]methylamine as previously described (22).

Materials. [^{14}C]galactose was obtained from the Radiochemical Centre, Amersham, England.

RESULTS

An alkaline shift increases resistance to killing by UV irradiation. Increased resistance to killing by UV irradiation may be a function of the level of an active SOS repair system (12). We therefore tested this parameter to study the correlation between pH_i and the functionality of the SOS system in two wild-type *E. coli* strains. The experimental system used was that described previously (22): cells were grown under pH-controlled conditions at pH 7.2 and shifted to pH 9 by titration. Under these conditions (22) (Fig. 1) the wild type showed a transient failure of the homeostatic response upon shift of pH_o, pH_i became alkaline, and concomitantly cell growth was arrested. The resistance to UV irradiation increased slightly after the increase in pH_i (Fig. 1). At the indicated time intervals, both before and after the pH_o shift, cells taken from the culture were irradiated for various time periods, and the T_{90} was determined. The T_{90}, which was 35 s before the shift, increased to about 40 s 5 min after the shift, reached 65 s at minute 10, and stayed constant during the next 15 min. After this period, T_{90} decreased back to the value observed before the pH_o shift. An almost identical behavior was observed in *E. coli* CS71, another K-12 strain, parent of the mutant DZ3 (data not shown).

When the mutant DZ3 was subjected to a similar pH shift (7.2 to 8.6), pH_i increased (Fig. 2) and cell division stopped (data not shown; see reference 22) for as long as the pH_o was maintained at the nonpermissive value. Accordingly, T_{90} increased from 50 s before the pH shift to about 80 s thereafter and remained at around this value as long as pH_o was nonpermissive (in parallel experiments not shown, this was tested for as long as 4 h). When pH_o was returned to 7.2, the permissive pH for growth (22), T_{90} decreased rapidly to the original value before the shift (Fig. 2).

An additional experiment suggested induction of a UV repair system: alkalinization of an *E. coli* C600 culture (pH_o 8.8) immediately before transformation with UV-irradiated T4 bacteriophage significantly increased the viability of the phage (data not shown).

Induction of galactokinase activity in *uvrA-galK* fusion plasmids. The results presented above suggest that the change in pH_i activates a UV repair system. To directly test whether this activation involves genes under SOS control, we used a plasmid (pA7) carying the *uvrA* promoter which is

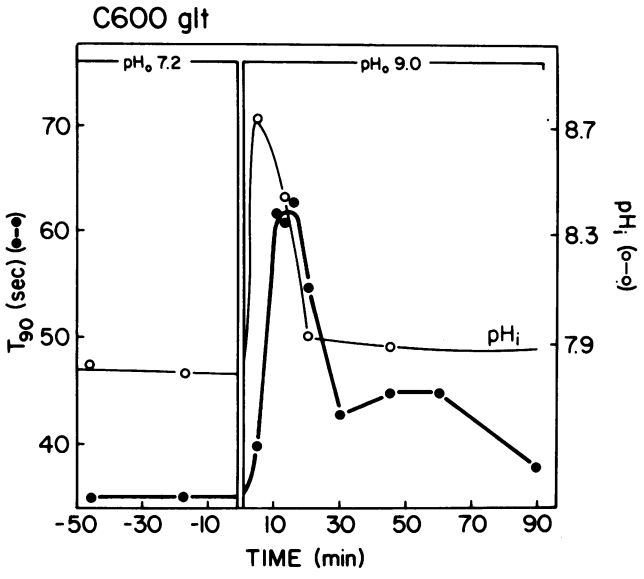

FIG. 1. Sensitivity of *E. coli* C600 to UV radiation upon shift to alkaline pH. *E. coli* C600-*glt* was grown in a chemostat under pH-controlled conditions as described in the text. Where indicated extracellular pH was increased by titration with KOH at a rate that gives a change of about 0.25 pH units/min and was maintained at this value throughout the experiment with a pH titrator as described in the text. At the indicated times 1-ml samples were taken, and cells were serially diluted in ice-cold medium. Sensitivity to UV radiation was determined as described in the text. Sampling of the cells (irradiation, dilution, and plating) takes about 5 min all together. At the time points in the figure cell handling was started.

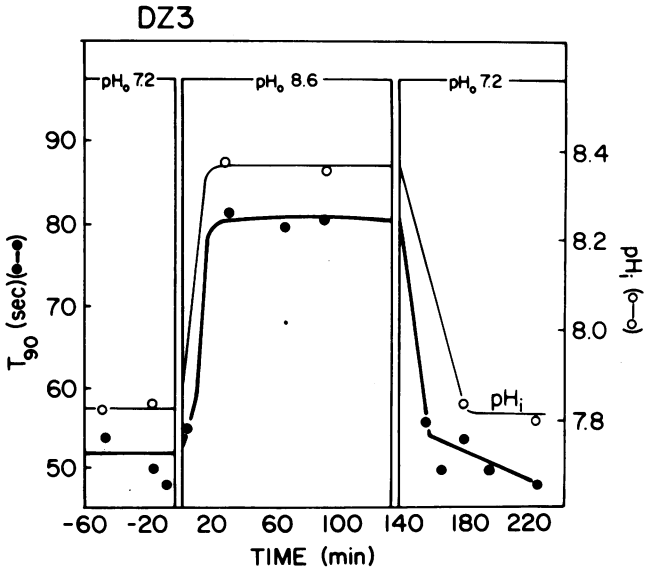

FIG. 2. Sensitivity of *E. coli* DZ3 to UV radiation upon changes in pH. The experimental details are described in the legend to Fig. 1, except that *E. coli* DZ3 was used and where indicated the extracellular pH was titrated back to 7.2 with HCl at a rate of about 0.3 pH units/min.

controlled by the SOS system and is fused to the structural gene of galactokinase (*galK*). Strains harboring such a plasmid display an up to 80-fold increase in galactokinase activity when treated with mitomycin C (Table 2), nalidixic acid, or UV light (1), agents known to induce the SOS response in *E. coli*.

When strain CS71 carrying the plasmid pA7 was subjected to a shift in pH_o from 7.2 to 8.6, cell division was transiently inhibited similar to the strain without the plasmid (data not shown) (22). An increment in galactokinase specific activity was detected immediately after the shift, and the specific activity increased thereafter to a level 7- to 10-fold the preshift level (in the specific experiment shown in Fig. 3 the specific activity increased fourfold) and stayed constant for at least 2 h. Similar results were obtained with the mutant DZ3 harboring pA7 (Table 2).

The level of SOS activation observed in CS71-derived strains was higher than previously reported in other strains (1). We therefore tested the effect of pH shift on a strain which could be activated by mitomycin C to a level similar to that reported previously. Qualitatively similar results were obtained when such a strain (C600-*glt*) carrying the plasmid pA7 was tested (Table 2). As expected, the activity of β-lactamase, an enzyme coded by the *bla* gene on the plasmid and not controlled by the SOS system, did not change upon shift of pH_o (Table 2). Moreover, in a strain harboring a fusion plasmid (pA8) which does not contain the SOS box of the *uvrA* promoter, the galactokinase activity was not induced by either mitomycin C or alkaline pH (Table 2). It is apparent that alkalinization of pH_o leads to activation of the *uvrA* promoter in a manner similar to activation by mitomycin C, and therefore the pH-mediated induction should also involve the *lexA* protein. To verify this suggestion, we introduced a *lexA3* mutation into *E. coli* CS71. This mutation encodes for a *lexA* protein which is resistant to *recA*-catalyzed cleavage (10), and therefore the resulting mutant CS71 *lexA* does not show the SOS response. When the pA7 plasmid was transformed into CS71 *lexA* there was no induction of galactokinase by either mitomycin C or shift in pH_o (Table 2). To test whether the pH-mediated induction also involves the *recA* protein, we transformed pA7 into a *recA* derivative CS71 strain. The resulting strain showed a very small (40 to 70%) but consistent increase in the galactokinase activity upon pH_o shift but not upon mitomycin treatment (Table 2). The results therefore suggest that there is a small *recA*-independent component in the pH-induced response.

TABLE 2. Induction levels of galactokinase in various *E. coli* strains upon shift of extracellular pH[a]

Strain	Galactokinase sp act with:			β-Lactamase activity at:	
	pH 7.2	pH 8.6	Mitomycin C	pH 7.2	pH 8.6
CS71(pA7)	20	160	1,700	0.4	0.4
DZ3(pA7)	40	120	1,100		
C600(pA7)	75	170	400	0.8	0.8
CS71(pA8)	26	34	37		
CS71 *lexA3*(pA7)	10	8	9		
CS71 *recA*(pA7)	27	45	25		

[a] The experimental details are as described in the legend to Fig. 3 except that the samples were assayed in triplicate. The values given for pH 7.2 are those measured 10 min before the pH shift. The values given for pH 8.6 are those measured 90 min after the shift. When mitomycin C was used (5 μg/ml) the experiment was done at a constant pH 7.2. Incubation time was 90 min. Galactokinase specific activity is expressed as in Fig. 3 (units per Klett units × 10^3). The β-lactamase activity in units per Klett unit.

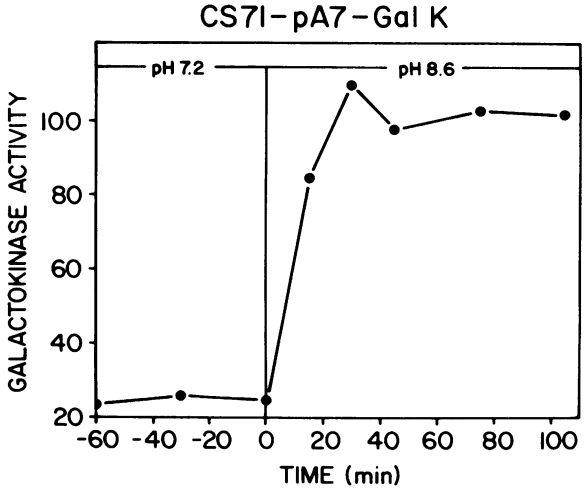

FIG. 3. Kinetics of induction of galactokinase in *E. coli* CS71(pA7) upon shift of extracellular pH. *E. coli* CS71(pA7) was grown in a chemostat under pH-controlled conditions as described in the text. Where indicated pH was increased with KOH at a rate that gives a change of about 0.2 pH units/min and was kept at this value throughout the experiment. At the indicated times samples were collected (in duplicate), and both CFU and galactokinase specific activity were determined. The galactokinase specific activity is expressed in units per Klett unit × 10^3 as described in reference 1 [(net counts per minute/total counts per minute) × (2,600/incubation time (minutes) × Klett units × 10^3)].

DISCUSSION

In this communication we present evidence that an increase in pH_i, upon failure of pH_i homeostasis, induces two responses: (i) resistance to killing by UV irradiation; (ii) increase in the expression of the *uvrA* promoter as monitored by the activity of the structural *galK* gene fused to the *uvrA* promoter.

The increased resistance to UV irradiation is transient in strains with a wild-type pH_i homeostatic response and permanent in a mutant incapable of regulating pH_i. In the latter, nevertheless, induction is reversed upon return to the permissive pH. In all cases tested the kinetics of induction correlated with inhibition of cell division and consequently with failure of pH homeostasis. It is tempting to conclude that increases in pH_i transiently or permanently induce the SOS functions responsible for the increased resistance to UV radiation. The transient nature of the response of the wild type to alkaline pH_o shift as opposed to the permanent response of DZ3 to a similar alkaline shift supports the contention that changes in intracellular pH rather than in extracellular pH induce the increased resistance to UV light. Both the "on" and "off" responses are very quick, probably within 10 to 20 min (the resolution time of the assay is 10 min). In this context we have also tested *lon* mutants (12, 19, 20) in which the off response is impaired. When shifted to the alkaline pH, a *lonA* strain, but not its parent, stops growing and filaments appear (V. Agmon, A. Cohen, S. Schuldiner, and E. Padan, unpublished observations), suggesting that, in these strains also, induction of the SOS systems by pH is irreversible.

A simpler and more direct measure of the induction of the *uvrA* gene was obtained with *uvrA-galK* fusion plasmids. Using this assay, it was shown that there is a rapid and significant induction of galactokinase activity upon alkalinization of the cytoplasmic pH. The level of β-

lactamase, an enzyme encoded by a plasmid gene which is not under control of the SOS system, does not change during the pH_i alkalinization. Since both genes *bla* and *uvrA* are carried on the same plasmid, different copy numbers of the respective genes cannot account for the difference in their response to changes in pH_i. It is concluded that pH_i leads to activation of *uvrA* by inducing the SOS system via the *lexA* protein. Indeed, when *galK* is fused to a noninducible *uvrA* promoter, galactokinase activity is not induced either by an agent such as mitomycin C or by pH. It still remains to be tested which other genes of the SOS system are induced by the pH shift.

Whatever the mechanism of the induction of the SOS system by pH_i, the phenomenon described opens up intriguing and exciting possibilities for the role of pH_i in the regulation of cell growth and metabolism. In addition to the fact that pH_i is an important parameter for almost every reaction in the cell, it may serve as a signal for dramatic changes in control processes in the cell. Thus, indications for its influence in spore germination (18) as well as in cell proliferation in tissue culture (17) and cell development after egg fertilization (7) and many other biological functions (see reference 14) have been documented. Also, it has been shown that the effect of pH_i on cell division in *E. coli* is not due to a general sensitivity of cytoplasmic proteins but to a more specific effect on a pH-sensitive step (22). In all cases a molecular link between pH_i and the given function is missing, and whenever explanations have been proposed they usually invoke a general pleiotropic effect of pH_i. The results presented in this study suggest that there is a pH-sensitive step in the pathway of activation of SOS response whether direct or via arrest of DNA replication or induction of DNA damage. Rapid, partial arrest of DNA synthesis has been observed upon increase of pH_i in the mutant DZ3 (22).

Even though we do not as yet have a proof that pH_i itself is indeed the signal for activation of the SOS system, it is tempting to speculate on a direct effect of pH_i on the *lexA* protein based on a recent report (11). In this report, Little showed that the purified *lexA* protein undergoes a cleavage at high pH (11). The time scale and the pH range of the experiments in vitro are different from those used in vivo for the induction of the SOS functions. In addition, the *recA*-independent fraction of the induction is very small. Nevertheless, studies are currently in progress to test the possible direct effect of pH_i on *lexA*. It may be that we are facing an example of a protein that senses changes in pH and transduces them to information that brings about specific modifications in the cell metabolism.

ACKNOWLEDGMENTS

We thank S. Handeli for bringing the paper by Little (11) to our attention and for helpful discussions. We also thank A. Openheim and D. Taglicht for valuable discussions and for critically reading the manuscript.

This work was supported in part by The Basic Research Foundation of the Israel Academy of Sciences and Humanities and by a grant from the United States-Israel Binational Science Foundation. J.B. is the recipient of an EMBO short-term fellowship.

LITERATURE CITED

1. **Backendorf, C., J. A. Brandsma, T. Kartasova, and P. van de Putte.** 1983. *In vivo* regulation of the *uvrA* gene: role of the "−10" and "−35" promoter regions. Nucleic Acids Res. **11**:5795–5810.
2. **Booth, I. R., and R. G. Kroll.** 1983. Metabolic control through transmembrane flux. Biochem. Soc. Trans. **11**:70–72.
3. **Citri, N., A. Kalkstein, A. Samuni, and N. Zyk.** 1984. Conformational adaptation of RTEM β-lactamase to cytoxitin. Eur. J. Biochem. **144**:333–338.
4. **Davis, G. D., and E. S. Mingioli.** 1950. Mutants of *Escherichia coli* requiring methionine or vitamin B_{12}. J. Bacteriol. **60**:17–28.
5. **Glassberg, J., R. R. Meyer, and A. Kornberg.** 1970. Mutant single-strand binding protein of *Escherichia coli*: genetic and physiological characterization. J. Bacteriol. **140**:14–19.
6. **Howard-Flanders, P., and L. Theriot.** 1962. A method for selecting radiation-sensitive mutants of *Escherichia coli*. Genetics **47**:1219–1224.
7. **Johnson, J. J., D. Epel, and M. Paul.** 1976. Intracellular pH and activation of sea urchin eggs after fertilization. Nature (London) **262**:661–664.
8. **Kihara, M., and R. M. Macnab.** 1981. Cytoplasmic pH mediates pH taxis and weak-acid repellent taxis of bacteria. J. Bacteriol. **145**:1209–1221.
9. **Krulwich, T. A., and A. A. Guffanti.** 1983. Physiology of acidophilic and alkalophilic bacteria. Adv. Microb. Physiol. **24**:173–214.
10. **Little, J. W.** 1983. The SOS regulatory system: control of its state by the level of *recA* protease. J. Mol. Biol. **167**:791–808.
11. **Little, J. W.** 1984. Autodigestion of *lexA* and phage λ repressors. Proc. Natl. Acad. Sci. USA **81**:1375–1379.
12. **Little, J. W., and D. W. Mount.** 1982. The SOS regulatory system of *Escherichia coli*. Cell **29**:11–22.
13. **Mount, J. D. W., K. B. Low, and S. J. Edmiston.** 1972. Dominant mutations (*lex*) in *Escherichia coli* K-12 which affect radiation sensitivity and frequency of ultraviolet light-induced mutations. J. Bacteriol. **112**:886–893.
14. **Nuccitelli, R., and D. W. Deamer.** 1982. Intracellular pH, its measurement, regulation and utilization in cellular functions. Alan R. Liss, Inc., New York.
15. **Padan, E., D. Zilberstein, and H. Rottenberg.** 1976. The proton electrochemical gradient in *Escherichia coli* cells. Eur. J. Biochem. **63**:533–541.
16. **Padan, E., D. Zilberstein, and S. Schuldiner.** 1981. pH homeostasis in bacteria. Biochim. Biophys. Acta **650**:151–166.
17. **Schuldiner, S., and E. Rozengurt.** 1982. Na^+/H^+ antiport in Swiss 3T3 cells: mitogenic stimulation leads to cytoplasmic alkalinization. Proc. Natl. Acad. Sci. USA **79**:7778–7782.
18. **Setlow, B., and P. Setlow.** 1980. Measurement of the pH within dormant and germinated spores of *Bacillus megaterium*. Proc. Natl. Acad. Sci. USA **77**:2474–2476.
19. **Walker, G. C.** 1984. Mutagenesis and inducible responses to deoxyribonucleic acid damage in *Escherichia coli*. Microbiol. Rev. **48**:60–93.
20. **Witkin, E. M.** 1976. Ultraviolet mutagenesis and inducible DNA repair in *Escherichia coli*. Bacteriol. Rev. **40**:869–907.
21. **Zilberstein, D., V. Agmon, S. Schuldiner, and E. Padan.** 1982. The sodium/proton antiporter is part of the pH homeostasis mechanism in *Escherichia coli*. J. Biol. Chem. **257**:3687–3691.
22. **Zilberstein, D., V. Agmon, S. Schuldiner, and E. Padan.** 1984. *Escherichia coli* intracellular pH, membrane potential, and cell growth. J. Bacteriol. **158**:246–252.
23. **Zilberstein, D., E. Padan, and S. Schuldiner.** 1980. A single locus in *Escherichia coli* governs growth in alkaline pH and on carbon sources whose transport is sodium dependent. FEBS Lett. **116**:177–180.