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Effect of *Salmonella typhimurium* Ferric Uptake Regulator (*fur*) Mutations on Iron- and pH-Regulated Protein Synthesis

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Fur is an important regulatory protein known to function in the presence of iron as a repressor of iron-controlled genes. It was recently discovered that Fur is also essential to *Salmonella typhimurium* for mounting an adaptive acid tolerance response (J. W. Foster, J. Bacteriol 173:6896–6902, 1991). Because little is known about the effect of Fur on the physiology of this enteric pathogen, a systematic two-dimensional polyacrylamide gel electrophoresis (PAGE) analysis was conducted to identify proteins whose synthesis is linked to iron levels. Mutations in the *fur* locus were identified and used to classify which proteins are controlled by Fur. Thirty-six proteins were overtly affected by iron availability, most of which were clearly under the control of Fur. Although most of the Fur-dependent proteins were under negative control, a significant portion (15 of 34) appeared to be under a form of positive control. Nine of the positively controlled proteins required Fur and iron for expression. However, Fur lacking iron was also required for the induction of six gene products. Surprisingly, not all iron-regulated proteins were controlled by Fur and not all Fur-dependent proteins were obviously regulated by iron status. Because *fur* mutants fail to mount an effective acid tolerance response, we made a comparative two-dimensional PAGE analysis of 100 total acid- and iron-regulated gene products. Production of most of these proteins was regulated by only one of the two stresses, yet a clear subset of seven genes were influenced by both acid and iron and were also controlled by *fur*. These proteins were also members of the acid tolerance response modulon. Consistent with the *fur* effect on pH-regulated protein synthesis, *fur* mutants lacked the inducible pH homeostasis system associated with the acid tolerance response. The results provide further evidence that Fur has an extensive impact on gene expression and cellular physiology and suggest an explanation for the acid-sensitive nature of *fur* mutants.

Iron is an extremely important element for biological systems (31). Consequently, organisms have evolved elaborate methods to acquire iron under limiting conditions (for reviews, see references 6 and 13). Bacteria, including *Escherichia coli* and *Salmonella typhimurium*, synthesize and excrete a variety of chelators or siderophores with extremely high affinities for iron. In addition to the enzymes required for siderophore synthesis, membrane proteins involved in recovering siderophore-iron complexes from the medium undergo transcriptional regulation by iron availability. The regulatory protein that mediates this regulation, designated Fur (ferric iron uptake regulator), binds Fe^{2+} and subsequently represses the expression of the many iron acquisition genes (6). Iron availability also controls the synthesis of a variety of toxins and other virulence determinants. The hemolysin of *Vibrio cholerae* (37), diphtheria toxin of *Corynebacterium diphtheriae* (10), Shiga toxin of *Shigella dysenteriae* (16), and Shiga-like toxin of *E. coli* (11) are examples of iron-regulated toxins. While considerable attention has focused on the ferric uptake regulator (*fur*) locus of *E. coli*, little work has been published regarding this regulatory gene in *S. typhimurium* or on iron metabolism in general in this organism (7, 8, 17).

As a facultative intracellular parasite, *S. typhimurium* is an excellent model system for studies of host-parasite interactions. During invasion, *S. typhimurium* enters a phagosome and subsequently encounters a wide array of hostile conditions, including reactive oxygen intermediates, antimicrobial peptides, acidic pH, and iron deprivation, among others. Successful intracellular parasites have developed a variety of strategies to avoid, disarm, or endure these lethal situations. *S. typhimurium*, for instance, possesses a variety of inducible defense mechanisms which are believed to enhance survival within the hostile phagosomal and phagolysosomal environments. One system under study, termed the acid tolerance response (ATR), enables adapted *S. typhimurium* to survive severe low-pH stress (20). We have recently discovered that strains of *S. typhimurium* harboring *fur* mutations fail to mount an effective ATR and as a result are extremely acid sensitive (19). The basis for the relationship between Fur and acid tolerance has not been established.

While the importance of iron metabolism to the cell is clear, there is no information available about iron-regulated proteins in *S. typhimurium* on a whole-cell basis. Consequently, a global study of the iron modulon will provide insight into the extent and nature of Fur-regulated gene expression and may offer clues to the acid-sensitive nature of *fur* mutants. With these goals in mind, we have conducted a two-dimensional polyacrylamide gel electrophoretic (PAGE) analysis of iron-regulated proteins in *S. typhimurium* and determined which are controlled by Fur. Based on similar investigations with other stress-regulated proteins, it was possible to integrate the iron regulation modulon within the general framework of stress management in *S. typhimurium*. The results indicate that Fur has a dramatic effect on protein synthesis and may act not only as a negative regulator, but in some instances as a positive regulatory element, either directly or indirectly. In addition, a significant overlap between iron-, Fur-, and pH-regulated protein synthesis was discovered.

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MATERIALS AND METHODS

Bacterial strains and culture conditions. *S. typhimurium* LT2 was used throughout this study. JF2023 is a derivative of LT2 that contains a spontaneous *fur* mutation. This and similar *fur* mutants were isolated by using an iron-regulated *iroA-lac* fusion. The *fur* mutants were identified as spontaneously arising, deregulated Lac^+ derivatives on MacConkey lactose medium supplemented with 60 μM $FeSO_4$. JF1819 [*atr*(Con)] is a constitutively acid-tolerant mutant (20). SF381 is an enterochelin-requiring mutant supplied by K. Sanderson, University of Calgary. The plasmids pABN203 and pMON2064 both contain the *E. coli fur*$^+$ locus and were kindly provided by J. B. Neilands (5) and R. D. Perry (36), with the permission of Monsanto Corporation, respectively.

The culture media used included E medium (38) and LB medium (14). Measurement of the adaptive ATR was detailed earlier (19). Briefly, cultures were grown in pH 7.7 minimal E with glucose under semianaerobic conditions to 10^8 cells per ml and then adapted to pH 5.8 for one doubling. Unadapted cultures were grown directly to 2×10^8 cells per ml at pH 7.7. Both cultures were then readjusted to pH 3.30 and incubated for 90 min. Viable cells were counted at 0 and 90 min.

Two-dimensional PAGE analysis of polypeptides. Two-dimensional PAGE was carried out as described previously (34). The first dimension was a pH 5 to 7 (right to left) isoelectric focusing gel containing 1.6% (pH 5 to 7) and 0.4% (pH 3 to 10) ampholytes (LKB-Pharmacia). The second dimension was an 11.5% polyacrylamide–sodium dodecyl sulfate (SDS) gel. Cells were grown in pH 7.0 minimal E medium (38) containing 0.4% glucose and supplemented with 120 μM $FeSO_4$ for iron-replete conditions. Iron-limiting conditions were achieved through the addition of an iron chelator, 100 μM diethylenetriamine pentaacetic acid (DTPA). In a second set of experiments, the iron chelator dipyridyl was used at 0.2 mM. Chelators were added to cultures that had attained a cell density of 10^8 cells per ml. After one to two doublings, the cells were labeled for 15 min with [^{35}S]methionine (^{35}S-Trans label; ICN) at 50 μCi/ml, harvested, and lysed in an SDS lysing solution as indicated by Spector et al. (34). Addition of DTPA or dipyridyl produced iron-limiting conditions sufficient to reduce the growth rate and induce expression of an iron-regulated *iroA-lacZ* fusion. The proteins indicated in Fig. 1 and Table 1 are those that were consistently observed to change over several experiments and with several *fur* mutants. Acidic and basic proteins are positioned to the right and left of each gel, respectively.

Other procedures. Transductions were performed with phage P22 HT 105/1*int* as described earlier (4, 26). The Tn*10*- and *lacZ*-directed Hfr strains were constructed as described by Chumley et al. (12) and Spector et al. (35). Transformations were performed by electroporation. β-Galactosidase production was assayed as described by Miller (28) and is expressed as micromoles per minute per optical density unit at 600 nm. Each strain was assayed in triplicate cultures.

Measurement of pH_i. The method of measuring the internal pH (pH_i) involved monitoring the distribution of radiolabeled weak acids or bases across the cellular membrane. The procedure was derived from that of Booth et al. (9). To determine intracellular water spaces, cells were grown in minimal medium containing 25 mM sucrose. At mid-log phase, 3 ml of cells was harvested and resuspended into 200 μl of culture supernatant. ^3H_2O and [^{14}C]sucrose were each added to 3,000 dpm/μl, a 5-μl sample was removed for determination of total counts, and the remainder was incubated at 37°C for 10 min (reaction mix A). Following incubation, duplicate samples (100 μl) were centrifuged through 50 μl of dibutyryl phthalate and 50 μl of silicone oil. The tubes were frozen at −70°C, and the cell pellet was sliced from the tube. The pellet was placed in a minivial containing 100 μl of 1% SDS for 5 min. Scintillation fluid was added, and the radioactivity in the series of vials was counted in an LKB 1219 scintillation counter. The total H_2O per cell pellet was calculated as dpm of ^3H_2O in the pellet/total dpm of ^3H_2O per μl of reaction mix. Extracellular H_2O was equal to dpm of [^{14}C]sucrose in the pellet/total dpm of [^{14}C]sucrose per μl of reaction mix. Intracellular water was equal to total H_2O minus extracellular H_2O.

An almost identical procedure was used to determine the distribution of a weak acid ([^{14}C]benzoic acid) or base ([^{14}C]methylamine), the difference being that the reaction mix (200 μl) contained 3,000 dpm of ^3H_2O and [^{14}C]benzoic acid or [^{14}C]methylamine. The formula used to calculate pH_i was as follows: $pH_i = \log [(\text{total acid in/total acid out}) (10^{pK} + 10^{pH_o}) - 10^{pK}]$.

RESULTS

Iron-regulated protein synthesis and overlap with other stress-regulated modulons. Two-dimensional PAGE analysis was used to define the relationship between iron-regulated protein synthesis and other stress modulons. A comparison of polypeptide profiles between cells grown under iron-replete versus iron-deficient (DTPA) conditions is presented in Fig. 1A and B, respectively. The quantitative levels of 41 proteins changed. Thirty-one were induced by apparent iron limitation, while 10 were repressed under the same condition. Table 1 lists the coordinates for each protein on a standard two-dimensional map of *Salmonella* proteins (34) and, in addition, indicates whether the protein was induced or repressed. To confirm that iron was the regulating metal, similar experiments were performed with dipyridyl as the iron chelator. Only five of the proteins regulated by DTPA chelation were not similarly affected by dipyridyl, indicating that they were regulated by a metal other than iron. Many of the 36 remaining proteins were regulated dramatically by iron availability, while others showed more modest changes. Comparisons with 10 other stress conditions revealed that 11 of the iron-regulated proteins were controlled in response to alternative environmental pressures. Nine of the dual-stress-controlled proteins responded to pH as the second stimulus, either as part of the pre-acid shock (pH 5.8) or post-acid shock (pH 4.5) components of the ATR. This indicates that a combination of iron and pH may serve as a regulatory signal for this subset of proteins. Although iron solubility varies with pH, these results reflect more than a simple relationship between iron availability and pH. Even though the solubility of $Fe(OH)_3$ is known to increase somewhat with increasing acidity, we found that the synthesis of most iron-regulated proteins remained insensitive to pH.

Identification of *Salmonella fur* mutants. The next phase of this study was to observe the effect of *fur* mutations on iron-regulated protein synthesis, but since the original *Salmonella fur* mutant is no longer available (17), we must provide proof that the mutations used in this study occur within the *fur* locus. The first step was to show that the mutations affect the expression of an iron-regulated locus. One such locus (*iroA*) was identified from a pool of *lacZ* insertion mutations constructed in our laboratory. Table 2

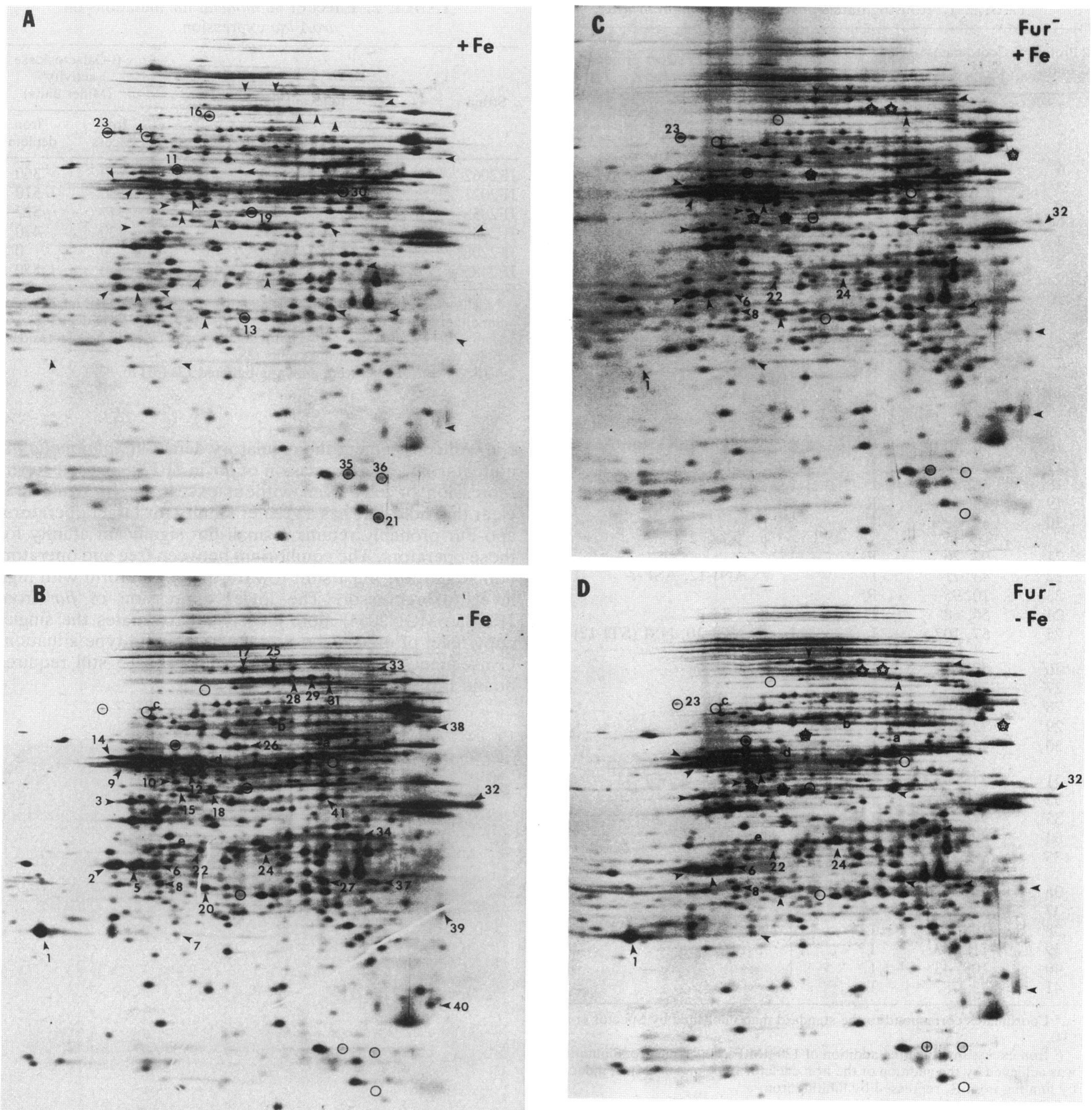

FIG. 1. Two-dimensional PAGE analysis of iron-regulated proteins in LT2 (A and B) and the *fur* mutant JF2023 (C and D). Panels A and C are the patterns obtained from cells grown in 120 μM $FeSO_4$. Panels B and D are from cells grown in 100 μM DTPA. (A and B) Arrowheads indicate iron-repressible proteins, and circles indicate iron-inducible proteins. Numbers refer to specific proteins (see Table 1). (C and D) Marked spots with numbers refer to proteins still regulated by iron; unmarked arrows and circles indicate proteins that are over- or underexpressed, respectively. Lowercase letters in panels B and D denote iron-independent proteins regulated by Fur. Acidic and basic proteins are located to the right and left, respectively. See Table 1 for details.

illustrates that *iroA* is induced by iron limitation and becomes constitutively expressed in a variety of putative *Salmonella fur* mutants. Figure 2 provides bioassay evidence that the *fur* mutants overproduce enterochelin. A ferric chloride-saturated filter paper disk placed next to a streak of fur^+ LT2 clearly showed repressed siderophore production as measured by cross-feeding of the enterochelin mutant SF381. No such repression was observed for JF2023 (Fur^-) or any of the other *fur* mutant strains tested.

By using the technique of Tn*10*-directed Hfr formation (12), the approximate location of these putative *fur* mutations was determined to be between 12 and 20 min on the *S.*

TABLE 1. Iron-regulated proteins of *S. typhimurium*

Protein no.	Coordinates[a] (X, Y)	Effect of limiting Fe[b]	*fur* expression[c]	Other stress proteins[d]
1	0, 52	I[e]		
2	7, 67	I	↑	
3	16, 78	I	↑	
4	21, 94	R	↓	ATR-2
5	18, 67	I	↑	
6	23, 67	I		
7	25, 50	I	↑	
8	30, 63	I[e]		
9	22, 85	I	↑	ANI-3
10	36, 81	I	↑	
11	36, 88	R	↓	
12	42, 88	I	↑	
13	53, 63	R	↓	
14	11, 86	I	↑	OXI-2
15[f]	42, 80	I	↓	
16	46, 98	R	↓	OXI-10, ATR-6, ASP-10
17	52, 102	I	↑	ATR-8
18[f]	46, 81	I	↓	
19	53, 81	R	↓	
20	47, 62	I	↑	
21	92, 26	R	↓	
22	43, 71	I		ANI-12, ASP-7
23	10, 95	R[e]		
24	56, 68	I[e]		
25	57, 102	I	↑	ATR-10, NSI (STI-12)
26[f]	52, 88	I	↓	
27[f]	74, 64	I	↑	
28[f]	67, 101	I	↓	
29	72, 101	I	↓	
30	73, 83	R	↓	
31	74, 99	I	↑	ATR-11, ASP-33
32	110, 78	I[e]		ATR-16
33	94, 102	I	↑	ATR-13
34	89, 72	I	↑	ATR-18
35	88, 37	R	↓	
36	93, 36	R	↓	PSI-12
37	93, 63	I	↑	
38[f]	108, 92	I	↓	
39	112, 53	I	↑	
40	109, 43	I	↑	
41	73, 78	I	↑	

[a] Coordinates correspond to the standard map published by Spector et al. (34).
[b] Iron excess involved the addition of 120 μM $FeSO_4$, while iron limitation was achieved by the addition of the iron chelator DTPA (100 μM). I, induced by limiting iron; R, repressed by limiting iron.
[c] Expression of Fur proteins in a *fur* mutant (JF2023). ↑, overexpression even with excess iron; ↓, underexpression or no expression even under derepressive conditions.
[d] Overlap with other stress-regulated modulons was identified by comparison with proteins induced by acid tolerance (20), acid shock (19), oxygen (OXI) or anaerobiosis (ANI), and starvation (STI) (34).
[e] These proteins were not induced with the addition of dipyridyl, a more specific iron chelator. They are regulated in response to an ion other than iron.
[f] These proteins appear to require deferrated Fur for induction during iron limitation.

typhimurium linkage map. Subsequent cotransductional analyses with *zbf-57*::Tn*10* placed this locus near *nag* at 15.5 min (33). This map position is consistent with that of *E. coli fur*. As a final proof, Table 2 reveals that cloned *fur*$^+$ from *E. coli* will complement the regulatory defect in *Salmonella fur* mutants. The overexpression of *fur* in JF2208 caused super-repression of *iroA* even without excess Fe^{3+}. While it is clear that holo-Fur has a greater affinity for target operators, apo-Fur probably retains a small but significant affinity for those operators. The equilibrium between free and operator-bound apo-Fur must shift toward the bound form with high levels of repressor. The lower expression of *fur* from JF2485(pMON2064) more closely approximates the single-copy level of expression present in the wild-type situation. Consequently, effective repression in JF2485 still requires bound iron.

TABLE 2. Effect of *Salmonella fur* mutations on *iroA-lac* expression

Strain	Relevant traits	β-Galactosidase activity[a] (Miller units)	
		Iron excess	Iron depleted
JF2062	*iroA-lacZ*	0	360
JF2021	*iroA-lacZ fur-1*	560	510
JF2058	*iroA-lacZ fur-7*	347	522
JF2059	*iroA-lacZ fur-8*	420	470
JF2208[b]	*iroA-lacZ fur-1*/pABN203 *fur*$^+$	0	0
JF2485[b]	*iroA-lacZ fur-1*/pMON2064 *fur*$^+$	0	570

[a] Cells were grown in minimal medium (pH 7.0) to 10^8 cells per ml, at which point either 60 μM $FeSO_4$ (iron excess) or 200 μM DTPA (iron depleted) was added. The culture was allowed to double prior to use. β-Galactosidase activity was measured in Miller units (28).
[b] JF2208 and JF2485 were grown in buffered LB (pH 8).

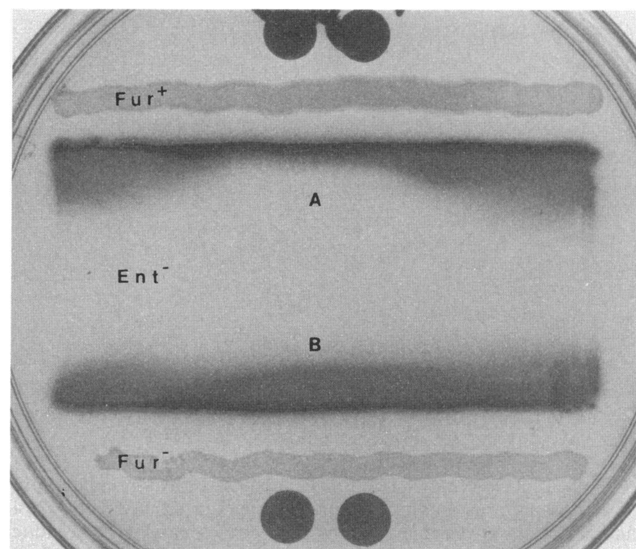

FIG. 2. Effect of *fur* on enterochelin production. E medium is low in free iron and will induce enterochelin production in a Fur$^+$ strain (LT2). This enterochelin crossfeeds the *ent* mutant (SF381) spread in the plate center. However, filter paper disks containing iron repress siderophore production by the Fur$^+$ strain, diminishing crossfeeding of the Ent$^-$ mutant. This is seen as a concavity in SF381 growth (A). In contrast, iron-containing filter paper disks did not repress enterochelin production in a *fur* mutant (JF2023) streak (B). To maximize growth contrast, SF381 contained a constitutive *lacZ* fusion and the medium contained the indicator 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside.

Effect of *fur* mutations on iron-regulated protein synthesis. Parallel to the two-dimensional PAGE study described above, a comparison of polypeptide profiles was made between wild-type LT2 and the *fur* mutant JF2023 (Fig. 1C and D). As expected, the production of a majority of iron-regulated proteins was clearly affected by the *fur* mutation. For the most part, if a protein was induced by iron limitation (iron repressible) in a wild-type cell, it was overproduced in the *fur* mutant under either high- or low-iron conditions. This was consistent with the classic role of Fur as a negative regulator (5). However, two striking results were noted that diverged from this norm. First, if a protein was normally induced by excess iron, it became uninducible in the *fur* mutant, suggesting that Fur may complex with iron to activate the transcription of some genes. Second, six Fur-dependent, iron-repressed proteins were underexpressed, not overexpressed, in the *fur* mutant. This is the opposite of what one would predict upon the loss of a negative regulator and indicated that deferrated Fur may be a positive regulator, either directly or indirectly, for a subset of iron-regulated genes.

A third discovery that resulted from studying the *fur* mutant was that not all of these proteins were Fur dependent. Seven proteins, indicated by their identification numbers in Fig. 1C and D, remained regulated by metal chelation with DTPA even in the absence of Fur. As noted above, five of these proteins must be induced in response to an ion other than iron, since a more specific iron chelator, dipyridyl, did not cause their induction (data not shown). However, two of the Fur-independent DTPA-induced proteins were also induced by dipyridyl. This result is suggestive of a second iron-sensing system in *S. typhimurium*, although we cannot unequivocally rule out induction of these two proteins through chelation of some ion other than iron.

Recent results from our laboratory indicate that Fur may influence the expression of some genes that do not respond overtly to fluctuations in iron concentration. For example, three pH-regulated genes that were not subject to obvious iron regulation were nevertheless rendered uninducible by acid pH in a Fur mutant (19a). Consequently, we expected to observe some proteins that were not detectably regulated by iron in the wild-type cell but were nevertheless underexpressed in the *fur* mutant. Five of these were observed and are indicated by letter in Fig. 1B and D.

***fur* mutations abolish ATR-specific inducible pH homeostasis.** Previous results noted above have shown that *S. typhimurium* requires Fur in order to effectively mount an ATR. The reason for this is not known, but the fact that several pre-acid shock ATR proteins were aberrantly expressed in *fur* mutants offers a possible explanation for their Atr$^-$, acid-sensitive phenotype. The pre-acid shock stage of the ATR induces a pH homeostasis system operative at external pHs below 4. Thus, at pH_o 3.3, adapted (pH 5.8) LT2 cells have a more alkaline pH_i than do unadapted cells (21). It seemed reasonable to suspect that *fur* mutants might be defective in ATR-specific pH homeostasis. This possibility was examined through determinations of pH_i for adapted (pH 5.8) and unadapted *fur* mutant cells at a variety of pH_o values. The results in Table 3 indicate no significant differences in pH_i at any pH_o examined above 4.0, suggesting that normal pH homeostasis mechanisms are operating appropriately. This was consistent with the results of an earlier study, showing that *fur* mutants do not exhibit general acid sensitivity (19). However, the data in the last two columns of Table 3 indicate that the adaptive pH homeostasis mechanism is inoperative in *fur* mutants. This deficiency will clearly result in an increased acid sensitivity below pH 4 while leaving survival above pH 4 unaffected. For comparative purposes, a constitutively acid-tolerant *atr*(Con)-*1* mutant (JF1819) was shown to possess enhanced pH homeostasis ability at a pH_o of 3.3 (pH_i of 5.7). The fact that the *fur* mutations do not affect pH homeostasis above pH 4.0 supports the view that these mutations specifically affect the ATR system and not classic pH homeostasis.

TABLE 3. Measurement of internal pH[a]

Strain	Relevant genotype	pH_i at pH_o of:					
		7.5	6.8	5.8	4.4	3.3 Unadapted	3.3 Adapted[b]
LT2	Wild type	7.87	7.6	7.1	6.1	4.4	5.0
JF2023	*fur*	7.8	7.6	7.0	6.1	4.1	4.2
JF1819	*atr-1*(Con)	7.8	ND[c]	ND	6.3	5.7	ND

[a] Internal pH was measured at 10 min after the shift to the indicated external pH (pH_o). The following weak bases or acids were used to measure internal pH: methylamine (pH 7.5), benzoic acid (pH 6.8, 5.8, and 4.4), and salicylic acid (pH 4.4 and 3.3).
[b] Cells were adapted at pH_o 5.8 for one doubling prior to a shift to pH_o 3.3.
[c] ND, not determined.

DISCUSSION

The results of these two-dimensional PAGE studies are summarized in Fig. 3. They indicate that Fur's contribution to gene expression is extensive. While the pleiotropic nature of *fur* mutations has been reported previously for *E. coli* (24, 25, 29), this polypeptide analysis dramatically illustrates the scope of Fur's impact on gene expression in *S. typhimurium* and the potential versatility with which it exerts control.

The pattern of Fur regulation can be explained in three ways. Control may be a direct effect of Fur on target genes, a cascade effect through intermediate regulators, or some combination thereof. The direct-effect model holds that Fur might exhibit four types of regulational control. When complexed with iron, Fur may function as either a negative (type A) or a positive (type B) regulator, depending upon the target locus. One of the more intriguing findings was that under iron starvation conditions, the regulator appeared to be necessary to activate the expression of several iron-repressed genes (type C). Lastly, Fur may act as a positive regulator of some genes in response to signals other than or in addition to iron (type D).

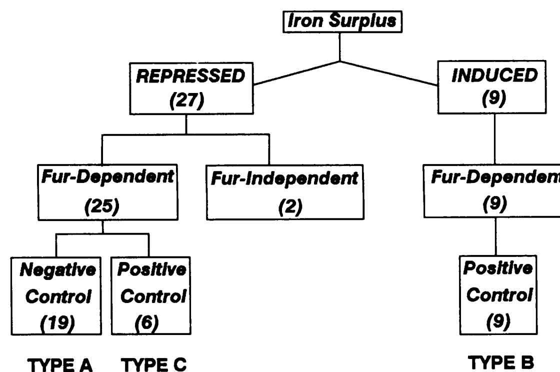

FIG. 3. Schematic representation of iron-regulated proteins. The number of proteins in each grouping is shown in parentheses.

That a single regulatory protein can either activate or repress different genes in response to a variety of signals is not without precedent. The cyclic AMP receptor protein has been shown to act as an activator and a repressor and possibly responds to different cyclic nucleotides (1–3, 22, 27, 33a, 39). With respect to Fur, Bagg and Neilands (5) have already shown that divalent cations other than Fe^{2+} can bind and activate the repressor in *E. coli*. Neiderhoffer et al. (29) provide evidence that the superoxide dismutase genes *sodA* and *sodB* are under negative and positive control, respectively, by Fur. It is perhaps reasonable to expect that a regulator as central to the cell as Fur will exhibit different types of control over the many genes that it regulates.

As an alternative to the direct-effect model, our results may also be explained by a cascade type of control. For example, if Fur negatively regulates the expression of a second negative regulator, the targets of that second regulator will appear to be under positive control by Fur. Cascade control by Fur in other systems has already been demonstrated and so should be considered likely in *S. typhimurium* (23). However, the definitive proof of one scenario or the other (or some combination) will require extensive in vitro analysis of Fur's interaction with target genes or with secondary regulators of the target genes. For example, it will be useful to examine type C and D loci for the presence or absence of a Fur box, the identified consensus DNA sequence to which iron-complexed Fur binds (15). Our data provide additional evidence at the whole-cell level of the complexity of Fur as a sensor-regulator molecule.

One goal of this study was to provide clues as to the nature of the Atr^- phenotype of *fur* mutants. It is significant that Fur influences the production of several pH-regulated gene products. The acid-sensitive Atr^- phenotype of *fur* mutants is likely a result of this influence. One or more of these low-pH- and Fur-controlled proteins may prove to be of integral importance to the ATR. The fact that *fur* mutants lack the ATR-specific inducible pH homeostasis system supports this theory. Additional studies are under way to identify which Fur-regulated protein(s) is integral to acid tolerance.

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