Global Iron-Dependent Gene Regulation in *Escherichia coli*:
A New Mechanism for Iron Homeostasis

Jonathan P McHugh1*, Francisco Rodríguez-Quiñones1*, Hossein Abdul-Tehrani2, Dimitri A Svistunenko3, Robert K Poole2, Chris E Cooper3 and Simon C Andrews1

1School of Animal & Microbial Sciences, University of Reading, Reading, RG6 6AJ, UK; 2Department of Molecular Biology & Biotechnology, The University of Sheffield, Sheffield, S10 2TN, UK; 3Department of Biological Sciences, University of Essex, Colchester, Essex, CO4 3SQ, UK

*These authors contributed equally to this work

Corresponding author: Dr Simon C Andrews,
School of Animal & Microbial Sciences, Whiteknights,
University of Reading, Reading, RG6 6AJ, UK
Tel. 00 44 (0) 118 9318463; Fax. 00 44 (0) 931 0180
Email  s.c.andrews@reading.ac.uk

Keywords: *nuo*, Fe-S clusters, respiration, *ompW*, nickel regulation, EPR, Fur, ribonucleotide reductase 2, iron transport, Ftr1p

Running title: Global Fe regulation in *E. coli*
ABSTRACT

Organisms generally respond to iron deficiency by increasing their capacity to take up iron and by consuming intracellular iron stores. *Escherichia coli*, in which iron metabolism is particularly well understood, contains at least 7 iron-acquisition systems encoded by 35 iron-repressed genes. This Fe-dependent repression is mediated by a transcriptional repressor, Fur (ferric uptake regulation), which also controls genes involved in other processes such as iron storage, the TCA cycle, pathogenicity and redox-stress resistance. Our macroarray-based global analysis of iron- and Fur-dependent gene expression in *E. coli* has revealed 14 new genes likely to specify at least three additional iron-transport pathways. Interestingly, a large group of ‘energy metabolism’ genes was found to be iron and Fur induced. Many of these genes encode iron-rich respiratory complexes. This iron- and Fur-dependent regulation appears to represent a novel iron-homeostatic mechanism whereby the synthesis of many iron-containing proteins is repressed under iron-restricted conditions. This mechanism thus accounts for the low iron contents of *fur* mutants and explains how *E. coli* can modulate its iron requirements. Analysis of $^{55}$Fe-labeled *E. coli* proteins revealed a marked decrease in iron-protein composition for *fur* mutants, and visible and EPR spectroscopy showed major reductions in cytochrome *b* and *d* levels, and in iron-sulfur cluster contents for the chelator-treated wildtype and/or *fur* mutant, correlating well with the array and quantitative RT-PCR data. In combination, the results provide compelling evidence for the regulation of intracellular iron consumption by the Fe$^{2+}$-Fur complex.
INTRODUCTION

Iron is an essential ‘minor element’ for most organisms, playing vital roles in many important biological processes including photosynthesis, N\textsubscript{2} fixation, methanogenesis, H\textsubscript{2} production and consumption, respiration, the TCA cycle, oxygen transport and DNA biosynthesis. However, despite the indispensability of iron, it is also potentially toxic due to its tendency to catalyze free-radical generation. In addition, the extremely poor solubility of the oxidized, ferric form leads to bioavailability problems (1). Organisms counter the difficulties posed by iron nutrition in a number of ways. One common mechanism involves the solubilization of extracellular iron, by reduction or chelation, followed by internalization via specific transporters. Another widespread approach is the deposition of intracellular iron stores within ‘ferritin’ molecules that can be subsequently utilized to abrogate the effects of iron restriction (1,2).

Iron metabolism in Escherichia coli K-12 is particularly well studied making it a model organism for investigations on iron-homeostatic processes. Like other bacteria, as well as fungi and some plants, it utilizes high-affinity extracellular ferric-chelators, called siderophores, to solubilize iron prior to transport (3). Ferri-siderophore complexes are taken up via specific outer-membrane receptors in a process that is driven by the inner-membrane potential and mediated by the energy-transducing TonB-ExbB-ExbD system. Periplasmic-binding proteins shuttle ferri-siderophores from the receptors to inner-membrane ABC transporters that, in turn, deliver the ferri-siderophores to the cytosol where the complexes are probably dissociated by reduction. E. coli has six known siderophore receptors (Cir, FecA, FepA, FhuA, FhuE, Fiu) providing specificity for several ferri-siderophores (and ferric dicitrate) of which only enterobactin and its derivatives are synthesized endogenously (4). It also possesses three ferri-siderophore periplasmic-binding
protein-dependent ABC-transporter systems, FecBCDE, FepBCDEFG and FhuBCD, and, like many other bacteria, can take up ferrous iron anaerobically via FeoB. In addition, *E. coli* contains three iron-storage proteins (Bfr, FtnA and FtnB) of which FtnA plays the major storage role (5).

Unsurprisingly, the iron-acquisition and -storage systems are regulated in response to iron availability. This regulation is mediated by the homodimeric repressor protein, Fur, which employs ferrous iron as co-repressor (4). There is evidence that the Fe$^{2+}$-Fur complex also represses genes (*cyoA*, *flbB*, *fumC*, *gpmA*, *metH*, *nohB*, *purR* and *sodA*) involved in various ‘non-iron’ functions (respiration, flagella chemotaxis, the TCA cycle, glycolysis, methionine biosynthesis, phage-DNA packaging, purine metabolism and redox-stress resistance) so it can thus be considered to be a global regulator (6-9). Fe$^{2+}$-Fur represses transcription by binding to a 19 bp sequence, designated the “iron box”, normally located near the Pribnow box of cognate promoters. Fur can also act as a transcriptional activator switching on genes encoding the iron-containing proteins aconitase A, Bfr, FtnA, fumarases A and B, succinate dehydrogenase and superoxide dismutase B (7, 10, 11). This activation appears to be indirect and seems to involve (at least in some cases) the Fe$^{2+}$-Fur repressed regulatory RNA, RyhB (11).

Here we use transcriptional profiling to extend the Fur modulon of *E. coli*. Over 100 Fe$^{2+}$-Fur-regulated genes were detected, most of which have not been previously reported. These include unknown genes potentially involved in iron acquisition. A large number of energy-metabolism genes, mainly encoding Fe-containing respiratory complexes, were found to be Fe$^{2+}$-Fur induced. This represents a major new functional category for inclusion within the Fur modulon. $^{55}$Fe-labelling studies and whole-cell spectroscopy showed that *fur* mutants are deficient in iron-containing proteins. Together, the data provide an explanation for the low iron
contents of fur mutants and reveal a new Fur-dependent mechanism for iron homeostasis.
MATERIALS AND METHODS

Bacterial Strains and Culture Conditions—E. coli strains were grown in Luria-Bertani (L broth) medium (12) at 37 °C and shaken at 250 rpm in an orbital shaker. Iron limitation was induced by inclusion of the ferrous iron chelator 2,2’-dipyridyl (dip) at 200 µM.

RNA Isolation, Preparation of Radio-labeled cDNA and Real-Time RT-PCR—Cultures of E. coli wildtype (MC4100), wildtype with dip and an E. coli fur mutant (H1941: MC4100 fur) were grown to an OD650nm of 1.0 (six replicates for each condition). A 1 mL sample from each culture was harvested by centrifugation and total RNA extracted using the Qiagen RNeasy® kit. RNA was treated with RNase-free DNase I (Promega). Each set of six replicate samples was pooled into two groups of three to control for slight growth and extraction variations. Pooled total RNA samples were then used as templates for production of 33P-labeled cDNA using random hexaprimers (Promega), and the labeled cDNA probes purified using G25-Sephadex Columns, as described by Sigma-Genosys. Quantitative RT-PCR was performed using an ABI 5700, the Sybr Green RT-PCR kit (Qiagen) and primers designed to amplify 50-80 bp fragments. Specificity was confirmed by electrophoretic analysis of the reaction products and by inclusion of template or reverse-transcriptase free controls. The template RNA samples were prepared separately from those used for macroarray analysis.

Macroarray Hybridisation and Scanning—Labeled cDNA was hybridized independently to two Panorama™ E. coli gene arrays (Sigma-Genosys) essentially as described by the manufacturer’s. Hybridized and washed arrays were then exposed to low intensity phosphoimaging cassettes (Molecular Dynamics) for 45 h and the cassettes were then scanned with a Phosphoimager SI system (Molecular Dynamics).
at 200 µm resolution. Filters were stripped prior to re-use as described by the manufacturer.

Data Analysis—The phosphoimager image files were analyzed using ImageQuant software (Molecular Dynamics). Pixel intensities for each spot were manipulated using Microsoft Excel. The 632 blank spots were used to calculate the background which was then subtracted from the 8580 gene-specific spots (each gene present in duplicate). Spots with intensities less than two standard deviations above mean background values were considered to display no significant expression. The intensity of each of the gene-specific spots within an individual array was normalized by expressing values as percentages of total gene-specific spot intensity. This allowed comparisons between array experiments. Array experiments were performed in duplicate, and average values calculated along with confidence levels (Student’s t test values).

Whole-Cell Spectroscopy—E. coli wild type (MC4100) and fur mutant (MC4100 fur) were grown aerobically in L broth, with or without 200 µM dip, to mid log (OD 0.5), late log (OD 1.0) and stationary phase (OD 3.0). Cells were harvested by centrifugation and 0.5 g of wet cells were resuspended in 10 mL of 0.1 M phosphate buffer (pH 7). Samples of 4 mL were oxidized with 0.005% (w/v) potassium ferricyanide and 0.005% (w/v) ammonium persulfate or were reduced with 0.005% (w/v) sodium dithionite. Spectra were then recorded at room temperature using a dual beam spectrophotometer over a wavelength range of 400-700 nm, with a reference wavelength of 500 nm (13). Cytochrome d and b-type cytochrome levels were quantified as previously described (14, 15). Prior to whole-cell EPR spectroscopy, cells were washed in 0.1 mM phosphate buffer (pH 7) containing 50 mM EDTA in order to remove Mn from the cell surface. Half of each sample was reduced by the addition of 0.005% (w/v) sodium dithionite followed by repetitive
freeze-thawing. The EPR spectra were measured at 10 K on a Bruker EMX EPR spectrometer equipped with an Oxford Instruments liquid helium system. A spherical high quality Bruker resonator SP9703 was used. The EPR samples were frozen in Wilmad SQ EPR tubes. Measurements were as follows: microwave power, 3.18 mW; microwave frequency, 9.46 GHz; modulation frequency, 100 kHz; modulation amplitude, 3 G; sweep rate, 3.58 G/s; time constant, 0.082 s. Protein concentrations were determined as previously described (16).

Native Gel Electrophoresis of $^{55}$Fe-labelled Soluble E. coli Proteins—E. coli cultures were grown in 5 ml of L broth containing $^{55}$FeCl$_3$ (~0.5 MBq/ml). Cells (5 OD$_{650nm}$ units) were harvested by centrifugation in the post-exponential phase and washed in saline at 4 °C. Soluble-cell extracts were then prepared by the spheroplast osmotic lysis method (17) with the following modifications. Tricine was used in place of Tris, dithiothreitol was omitted, 0.1% Triton X-100 was included at the lysis stage and glycerol was added post-lysis to a final concentration of 5%. Soluble-cell extracts were electrophoresed in native-acrylamide gels containing 0.1% Triton X-100 and Tricine in place of Tris. Gels were then dried under vacuum and autoradiographed with Kodak Biomax MS film.
RESULTS AND DISCUSSION

Identification of Fe$^{2+}$-Fur Regulated Genes by Transcriptional Profiling—Genomic transcriptional profiling was used to identify genes regulated by both Fe and Fur (see Methods). The transcription profile of MC4100 (wild type) grown in rich broth was compared with those of both the wild type grown with an Fe$^{2+}$ chelator (dip) and the fur mutant (MC4100 fur) grown without chelator. Samples were harvested at an OD$_{650nm}$ of 1.0, corresponding to early post-exponential phase. No major growth differences were observed for the three experimental conditions. $^{33}$P-labeled cDNA was prepared from the RNA samples using reverse transcriptase and random hexa-oligonucleotides, and was hybridized to *E. coli* Panorama Macroarrays. Each array experiment was performed in duplicate using pooled RNA samples prepared from three identical cultures. Duplicate experiments gave good reproducibility with correlation coefficients of 0.97. Comparison of the L broth versus L-broth plus dip, or wild type versus fur mutant, gave lower correlation coefficients (0.95 and 0.90).

Since members of the Fur modulon are regulated by iron and Fur in conjunction, only those genes that were $\geq$twofold regulated by both dip and the fur mutation are considered further here. Accordingly, 101 genes were found to be regulated by the Fe$^{2+}$-Fur complex of which 53 were repressed and 48 induced. These genes fall into three major categories: iron metabolism (Table I); energy production (Table II); and miscellaneous/unknown (Table IV).

Iron Metabolism: Potential Novel Iron Transporters—Reassuringly, most of the known iron-acquisition genes were induced by both the chelator and the fur mutation (Table I) validating the experimental procedure. The enterobactin biosynthesis (*entA*-F) genes were amongst the most highly de-repressed (average $\sim$21-fold) genes (Table I), presumably to ensure that energy is not needlessly squandered on enterobactin production during conditions of iron sufficiency. In contrast, the ferric-enterobactin
uptake genes \((fepABCDEG)\) were weakly de-repressed (average ~twofold) indicating that enterobactin production systems are more strongly controlled by iron than the ferri-enterobactin acquisition apparatus. As previously observed, the \(fecIRABCDE, feoAB, fhuE, fhuF\) and \(cir\) genes involved in ferric-dicitrate, ferrous iron, ferri-coprogen/rhodotorulic acid, ferrioxamine B and ferric-dihydroxybenzoate utilization were all repressed by the \(\text{Fe}^{2+}\)-Fur complex, as were the \(tonB\) and \(exbBD\) genes required for energy-dependent ferrisiderophore transfer across the outer membrane. However, the \(fhuACDB\) operon, specifying the ferric-hydroxamate uptake apparatus, was not significantly affected by the chelator or \(fur\) mutation although it is known that \(fhuA-lacZ\) transcriptional fusions are \(\text{Fe}^{2+}\)-Fur regulated (19). The reason for this discrepancy is unclear, but could be related to growth-phase effects. The \(suf\) operon, which probably functions in iron-sulfur cluster assembly during iron starvation and redox stress (20), was also \(\text{Fe}^{2+}\)-Fur repressed (average of fivefold), as previously indicated (21). However, the \(iscSUAB-hscBA-fdx\) cluster, which encodes genes with a housekeeping role in iron-sulfur cluster assembly, was not \(\text{Fe}^{2+}\)-Fur controlled (not shown). Appropriately, the \(bfrd\) gene encoding a ferredoxin thought to be involved in iron release from bacterioferritin, was de-repressed (22) whereas the \(finA\) gene specifying the iron-storage protein, ferritin A, was repressed by the chelator and \(fur\) mutation, as previously observed (5,11). A significant absentee from the list of genes in Table I is \(bfr\), coding for bacterioferritin. Although this gene is known to be \(\text{Fe}^{2+}\)-Fur induced, its expression is RpoS dependent and so is restricted to the stationary phase (unpublished observations). Thus, the array data are generally consistent with numerous previous expression studies on the Fur-regulated components of iron metabolism.
The array analysis enabled the identification of 14 ‘unknown’ genes with probable functions in iron acquisition (Table I). These were initially recognized either because of their chelator- and fur-dependent expression or by their chromosomal co-location with such genes. They are organized into 6 clusters (boxed in Table I), of which the largest (ybiM-ybiLXI-ybiJ) consists of 5 co-polar genes encoding: YbiM and YbiJ, two putative-periplasmic/exported proteins that are related to each other, but not to any other E. coli protein; YbiL (or Fiu), a probable TonB-dependent outer-membrane receptor previously shown to be involved in Fe$^{3+}$-dihydroxybenzoylserine and –dihydroxybenzoate utilization (23); YbiX, a homologue of a protein encoded by an Fe-repressed Pseudomonas aeruginosa gene (piuC) thought to be involved in iron uptake (24); and YbiI, a probable C4-Zn finger protein of unknown function. The above suggests that the Fiu-mediated Fe-uptake system is more complex than hitherto believed.

The second cluster (ycdN-ycdOB) consists of three copolar genes encoding: YcdN, a homologue (24% amino acid sequence identity) of the high-affinity ferrous iron transporter (Ftr1p) of yeast; YcdO, a potential exported lipoprotein of unknown function; and YcdB, another potentially exported protein of unknown function. A ‘twin arginine transporter’ (tat) motif was previously identified in the N-terminus of YcbB (25), and a similar motif is present in the N-terminus of YcdO. This suggests, as for many other tat-exported proteins, that these proteins could possess prosthetic groups inserted prior to export to the periplasm. Homologues of the ycdNOB genes are found co-located in the chromosomes of at least 7 other bacteria indicating that these 3 genes form a functional unit. YcdB bears homology to STY2683 of Salmonella typhimurium, a putative iron-dependent peroxidase. By analogy with the yeast Ftr1p system (26), we speculate that YcdN acts as a ferrous iron transporter, and
that YcdO and YcdB act together as a novel periplasmic iron oxidase or reductase (this possibility is currently being tested). The third cluster consists of three genes, *yddAB-pqqL*, that appear to form an operon encoding: YddA, which is homologous to ABC transporters; YddB, homologous to TonB-dependent outer-membrane receptors; and PqqL, a potential Zn peptidase (*pqqL* is also induced by iron restriction in *Pasteurella multocida*; 45). These genes are likely to specify a new 3-component iron-uptake system. The fourth cluster is also likely to represent a newly identified iron-uptake pathway. It consists of two divergently-arranged genes encoding: YcnD, a probable TonB-dependent outer-membrane receptor; and YncE, which is predicted to be a pyrolo-quinoline quinone containing periplasmic oxidase. Note that all four of the above Fe$^{2+}$-Fur repressed gene clusters are associated with predicted Fur boxes.

The fifth locus consists of a single gene (*ydiE*) that is related (36% identity at the amino acid sequence level) to *hemP*, a gene that forms part of the heme utilization operon (*hemPRST*) of *Yersinia enterocolitica* (27). The specific function of *hemP* is uncertain. The *ydiE* gene is associated with a well-predicted Fur box and its homologue (*hemP*) in *S. typhimurium* is Fur repressed (28), supporting the Fe$^{2+}$-Fur dependent regulation observed here. The final locus also comprises a single gene, *yqjH*, homologous (28% identity at the amino acid sequence level) with the siderophore-utilization gene (*viuB*) of *Vibrio cholerae*. *yqjH* has an appropriately positioned potential Fur box (29) consistent with its Fe$^{2+}$-Fur dependence.

**Energy Metabolism: Control of ‘Iron-Rich’ Proteins**—Unexpectedly, a large number of genes encoding proteins involved in energy metabolism were found to be Fe$^{2+}$-Fur regulated (Table II). Of those genes and operons listed in Table II, only *cyoA* and *gpmA* have previously been reported to be Fe$^{2+}$-Fur controlled (8, 6). Most (36) were induced by the Fe$^{2+}$-Fur complex of which 32 encode iron-containing respiratory
complexes associated with a total of 148 iron atoms (per subunit). We speculate that this Fur-dependent control of iron-containing respiratory proteins represents a newly-recognized iron homeostatic mechanism whereby the production of a sub-set of iron proteins is regulated according to iron availability. Such a mechanism would allow the cellular demand for iron to be reduced under iron-restricted growth conditions, enabling available iron to be utilized more economically and helping to ensure that production of Fe-requiring proteins does not exceed iron availability. Partially consistent with the data in Table II, previous work has shown that the anaerobic expression of cydA, cyoA, narG and frdA is 2- to 14-fold reduced by dip (note that for cyoA and cydA this effect required an fnr background) (30). However, in contrast to the findings presented here, this effect appeared to be Fur-independent (the iron-dependent regulator was not identified; 30).

Nearly all of the Fe$^{2+}$-Fur induced genes in Table II require the anaerobic regulator, Fnr, and anaerobiosis for full induction (31). Although the growth conditions used here were aerobic, O$_2$ tensions are very low during late-log growth in rich broth (32) and thus could favor Fnr-dependent expression. It is also possible that anaerobic conditions were introduced at the sample harvesting stage. A potential complication is the possible inactivation of Fnr by the iron chelator and consequent down regulation of the Fnr-dependent genes in Table II (30). However, such an effect would not be anticipated for the fur mutant and so would not explain both the dip and the fur expression effects shown in Table II. The Fe$^{2+}$-Fur induced genes listed in Table II are not essential and the observed reductions in their expression during aerobic growth would not be expected to lead to a major growth defect. Indeed, although fnr mutants are unable to express anaerobic respiratory complexes, they retain the ability to grow both anaerobically (via fermentation) and aerobically.
The induction of expression by the Fe$^{2+}$-Fur complex is likely to be indirect since for most of the Fe$^{2+}$-Fur induced genes (Tables I, II and IV), there appears to be no associated Fur boxes. It is probable that the recently-identified Fur-dependent regulatory RNA molecule, RyhB, acts as the direct regulator of these genes (11). It is noteworthy that not all Fe-protein encoding genes appear to be induced by Fe$^{2+}$-Fur. This may reflect the high importance of some Fe-proteins, such as the aerobic ribonucleotide reductase (NrdAB) required for DNA biosynthesis. It is also interesting to note that the expression of \textit{gpmA} (encoding phosphoglycerate mutase in the glycolytic pathway) and \textit{gltA} (encoding the TCA cycle enzyme, citrate synthase) is repressed by the Fe$^{2+}$-Fur complex (Table II), whereas \textit{acnA} (encoding the TCA cycle enzyme aconitase A that converts citrate into isocitrate) is known to be induced by Fe$^{2+}$-Fur, suggesting that \textit{E. coli} may respond to iron restriction by producing citrate to mediate iron uptake. Expression of the citrate synthase gene (\textit{prpC}) is also Fur repressed in \textit{Shewanella oneidensis} (44).

Quantitative RT-PCR was used to confirm the Fe$^{2+}$-Fur dependent expression of 12 iron-regulated genes (Table III). Although the directions of regulation were identical for the RT-PCR and array data, the degree of regulation varied considerably and was generally greater for the RT-PCR analysis, presumably reflecting the higher quantitative precision obtained with RT-PCR. Control experiments with \textit{polA} (encoding DNA polymerase I) showed no significant Fe$^{2+}$-Fur effect and thus matched the DNA array data.

\textbf{Cellular Iron-Protein Composition is Fur Dependent}—The observed Fe$^{2+}$-Fur dependent expression of Fe-proteins is consistent with the 70% reduction in iron levels caused by the \textit{fur} mutation (5). Part (50%) of this reduction in iron content is due to low levels of FtnA iron stores, and it was suggested that the residual (20%) reduction in iron content is due to decreased expression of Fe-proteins (5).
suggestion is supported by the array data presented in Table II. In order to determine
whether the fur mutation does indeed lead to significantly decreased levels of cellular
Fe-proteins, a comparison of the soluble Fe-protein composition of wildtype and fur
mutants grown in L broth with $^{55}$Fe was performed (Fig. 1). This analysis confirmed
that the fur mutant does indeed have a much lower level (~ 2 fold, as estimated by
densitometric analysis of the autoradiograph presented in Fig. 1) of Fe-proteins than
the wildtype supporting the notion that Fur controls cellular levels of Fe-proteins as
suggested here and elsewhere (5, 11, 30).

Whole-cell spectroscopy reveals that cytochrome bd and Fe-S protein levels are Fur
and/or iron regulated—To provide further evidence for the Fe$^{2+}$-Fur control of Fe-
protein levels, whole-cell spectroscopy was used to measure the effect of dip and/or
the fur mutation on the relative abundances of cytochromes and Fe-S proteins (Figs. 2
& 3). Visible difference spectroscopy measurements revealed that the cytochrome d-
dependent signal at 630 nm is decreased by 3-fold (from 0.06 to 0.02 nmol/mg
protein) by dip or the fur mutation (Fig. 2), which correlates well with the 2.7-fold
decrease in expression observed for the corresponding genes (cydAB; Table II). The
signal at 560 nm (Fig. 2) is due to overlapping $\alpha$-bands from $b$-type cytochromes (33),
primarily cytochrome $b_{562}$ of the cytochrome $bo_3$ quinol oxidase, the low-spin
cytochrome $b_{558}$ of the cytochrome bd quinol oxidase, and heme $b_{556}$ of succinate
dehydrogenase (34). The 560 nm band is 2.9-fold reduced (from 0.2 to 0.07 nmol/mg
protein) by dip or the fur mutation which is consistent with the expression data for
both the cydAB and cyo genes (Table II).

Low-temperature electron-paramagnetic-resonance (EPR) spectroscopy of
whole cells was used to compare the levels of fully assembled Fe-S containing
proteins. When the cells are frozen as prepared, the Fe-S proteins are found mainly in
the oxidized state (Fig. 3A). In this state the major EPR-detectable species is the 3Fe-
4S cluster that can be found in, for example, succinate dehydrogenase and fumarate reductase. The content of the 3Fe-4S clusters in the fur mutant is ~fourfold lower than in the wt sample (likely to be derived from fumarate reductase, rather than succinate dehydrogenase; Table 2). This correlates well with the 4.2-fold decrease in expression of the fumarate reductase operon (frdABCD) in the fur mutant. The macroarray data showed no significant effect of dip or the fur mutation on sdhCDAB expression and this lack of Fe^{2+}-Fur response is supported by RT-PCR on sdhB and sdhC (not shown). However, preliminary array studies (not shown) using ‘minimal’ (as opposed to ‘rich’) medium showed a clear iron-dependent expression of the sdh operon, as reported previously (11, 36). A signal at g=4.3, arising from iron in a “rhombic” conformation, is also present in the oxidized samples (Fig. 3B). The most likely species responsible for this signal is a mononuclear non-heme iron center (35). This feature was sevenfold higher in the fur mutant. The identity of this species is unknown but it could correspond to a ‘labile free-iron species’, as previously observed in fur mutants (37).

Other Fe^{2+}-Fur regulated genes—Forty six genes, from various ‘other’ functional categories, were also Fe^{2+}-Fur controlled (Table IV). Of these, only nrdH, sodB, and yhhY have previously been reported to be Fe^{2+}-Fur regulated (8, 39). Of particular note is the apparent Fe^{2+}-Fur induction (average 2.1 fold) of the Ni^{2+}-transport genes (nikA-R; 40). Since all known nickel-containing proteins in E. coli are hydrogenases, which also require iron, it is not surprising that Ni^{2+} transport is induced by the Fe^{2+}-Fur complex. Of the four hydrogenase operons, the array data show only that of hydrogenase 2 (hybOA-G) is iron and Fur regulated (1.4- and 1.7-fold induced, respectively; data not shown). However, this effect is relatively weak and how it is mediated is unclear. Interestingly, a heat-shock gene cluster (hslTS-yidE) was Fe^{2+}-Fur repressed (average 3.7 fold), whilst a group of cold-shock genes (cspI, cspB and
cspF) within a phage gene cluster was induced (average 2.7 fold) by the Fe$^{2+}$-Fur complex. This suggests that temperature-shock-dependent gene induction may be partly due to transient changes in intracellular iron availability or Fe$^{2+}$-Fur stability. The nrdHIEF operon encoding a non-essential iron-containing ribonucleotide reductase (41) appears to be Fe$^{2+}$-Fur repressed (average 2.6 fold) which is supported by previous work showing Fe$^{2+}$-Fur repression of nrdH (8). This indicates that this isoenzyme (there are three alternative ribonucleotide reductases in E. coli) may have a role under iron-restricted conditions. The Fe$^{2+}$-Fur induced ompW gene encoding an outer-membrane of uncertain function is also induced by iron in Pasteurella multicauda (45), although it is repressed by iron in S. oneidensis (44).

In addition to bfr, fhuABCD and sdhCDAB, there are several other known Fe$^{2+}$-Fur regulated genes (acnA, flbB, fumA, fumB, fumC, purR, nohB, sodA, ygaC and yhhX) that were not affected by dip and Fur in this study. The reasons for this are probably related to the growth conditions employed here which are unlikely to favor expression of all Fur-controlled genes. For instance, acnA expression is $\sigma^3$ and SoxSR dependent and so requires stationary-phase and redox-stress for maximum induction (42), and our preliminary data (not shown) suggest that the PurR-controlled purine regulon is iron-dependent in minimal (rather than rich) medium. Note that the Fe$^{2+}$-Fur regulated promoter originally identified upstream of yhhX (8) appears to be specific for ryhB (11), which would explain its lack of Fe$^{2+}$-Fur control in this work.

**General relevance**—This study has revealed that, despite much previous work, there is still a great deal to be discovered concerning iron metabolism in E. coli K-12. A major new role for Fur in iron homeostasis has been demonstrated in which Fur mediates the control of cellular iron-protein levels in response to iron availability. This new mechanism is relatively simplistic in that it only involves iron-responsive gene regulation. It is currently unclear how widespread this mechanism is but, given
its simplicity, it is possible that it could be common, especially amongst organisms with the capacity to globally regulate gene expression in response to iron. Recent global transcriptional profiling studies on the effects of iron restriction and/or fur inactivation provide some evidence for iron-regulation of Fe-protein genes in other bacteria. In *Bacillus subtilis* (43) several cytochrome systems (e.g. *cydABCD*) and aconitase (*citB*) were reported to repressed by iron limitation. In *S. oneidensis*, a *fur* mutation resulted in reduced expression of genes encoding proteins (cytochrome *c* oxidase, cytochrome *c* maturation protein B, cytochrome *b*$_{561}$) involved in electron transport (44), and in *P. multocida*, genes encoding proteins (e.g. fumarate reductase, dimethylsulphoxide reductase, putative iron-sulfur protein, NapF, formate dehydrogenase) involved in energy metabolism and electron transport were reduced during iron restriction by 2 to 6 fold (45). In *P. aeruginosa*, 87 genes were repressed by iron restriction, although their identities have not yet been reported (46).

**Acknowledgements**—This work was supported by the BBSRC through a PhD studentship to JM and project grants to SCA and to RKP, by the Wellcome Trust through a grant to CEC and by the Iranian Government through a PhD studentship to HAT. We thank K Hantke for the provision of strain H1941.
**TABLE I.**

Iron and Fur regulation of genes involved in iron metabolism.

| Gene1,5          | Blattner No. | Description8 | Level (ppm) | Dip3,4 | fur | Fur site6 Score | Pos7. |
|------------------|--------------|--------------|-------------|--------|-----|----------------|-------|
| fhuACDB         | b0150-3     | Fe-hydroxamate transport | 1,202       | +1.23  | +1.38 | 17.0(14)       | -39   |
| entD            | b0583       | enterobactin biosynthesis | 13          | +4.99  | +15.58 |                |       |
| fepA            | b0584       | Fe-enterobactin transport | 558         | +3.87  | +7.05  | 16.1(12)       | -160  |
| fes             | b0585       | Fe-enterobactin utilization | 99         | +6.54  | +8.14  | 16.1(12)       | -137  |
| entF            | b0586       | enterobactin biosynthesis | 26          | +14.54 | +6.90  |                |       |
| fepE            | b0587       | Fe-enterobactin transport | 182         | +1.03  | +1.19  |                |       |
| fepC            | b0588       | enterobactin biosynthesis | 76          | +1.64  | +1.96  |                |       |
| fepG            | b0589       | Fe-enterobactin transport | 76          | +1.39  | +1.82  |                |       |
| fepD            | b0590       | Fe-enterobactin transport | 77          | +1.47  | +1.61  | 15.0(16)       | -13   |
| fepB            | b0591       | enterobactin export | 32          | +2.36  | +3.48  |                |       |
| entC            | b0592       | Fe-enterobactin transport | 204         | +2.70  | +2.26  | 18.8(15)       | -74   |
| entE            | b0593       | enterobactin biosynthesis | 14          | +36.73 | +47.02 | 21.6(15)       | -99   |
| entF            | b0594       | enterobactin biosynthesis | 48          | +26.90 | +31.59 |                |       |
| entB            | b0595       | enterobactin biosynthesis | 7           | +41.73 | +41.00 |                |       |
| entA            | b0596       | enterobactin biosynthesis | 50          | +9.60  | +17.32 |                |       |
| ybdB            | b0597       | enterobactin metabolism? | 36          | +5.62  | +9.64  |                |       |
| ybdC            | b0802       | exported/periplasmic protein? | 21          | +1.89  | +2.13  |                |       |
| ybdO            | b0803       | C4-type Zn-finger, dksA/traR-like | 65          | +1.49  | +1.61  |                |       |
| ybiL            | b0804       | like piuC (Fe-uptake, *P. aeruginosa*) | 29          | +22.64 | +34.81 |                |       |
| ybiN            | b0805       | fhu, TonB-dependent receptor | 98          | +30.72 | +37.96 | 19.1(14)       | -99   |
| ybiM            | b0806       | exported/periplasmic protein? | 11          | +1.20  | +1.67  |                |       |
| ycbN            | b1016/7     | like yeast Fe permease (FTR1) | 12          | +1.20  | +2.04  | 18.4(15)       | -51   |
| ycdO            | b1018       | lipoprotein? | 90          | +2.21  | +2.81  |                |       |
| ycdB            | b1019       | lipoprotein? Fe dependent peroxidase? | 291         | +2.60  | +2.48  |                |       |
| ycdC            | b1102       | Fe-hydroxamate transport | 355         | +3.42  | +1.76  |                |       |
| ycdO            | b1125       | Fe-siderophore & B12 transport | 243         | +1.88  | +2.04  | 16.0(12)       | -48   |
| nycD            | b1451       | TonB-dependent receptor? | 644         | -1.34  | -1.03  | 18.9(15)       | -139  |
| nycE            | b1452       | PQQ-containing periplasmic oxidase? | 76          | +7.78  | +18.05 | 18.9(15)       | -85   |
| pqqL            | b1494       | Zn-peptidase? | 102         | +4.29  | +2.31  |                |       |
| yddB            | b1495       | TonB-dependent receptor? | 173         | +1.22  | +1.01  |                |       |
| yddA            | b1496       | ABC-transporter? | 102         | +2.43  | +2.81  | 17.6(15)       | +6    |
| sufE            | b1679       | Fe-S formation (in FhuF) | 34          | +4.45  | +5.46  |                |       |
| sufS/csdB       | b1680       | Fe-S formation (in FhuF); IscS-like | 15          | +5.11  | +6.39  |                |       |
| suD             | b1681       | Fe-S formation (in FhuF) | 21          | +11.22 | +9.66  |                |       |
| sufC            | b1682       | Fe-S formation (in FhuF); ABC motif | 74          | +4.10  | +2.70  |                |       |
| sufB            | b1683       | Fe-S formation (in FhuF); ABC motif | 241         | +4.22  | +2.89  |                |       |
| sufA            | b1684       | Fe-S formation (in FhuF); IscA-like | 71          | +4.46  | +2.20  | 18.3(15)       | -47   |
| ydeE            | b1705       | like hemP (haem uptake) | 15          | +2.91  | +8.12  | 17.3(15)       | -91   |
| ftnA            | b1905       | Fe storage | 150         | -4.07  | -3.51  |                |       |
| cirA            | b2155       | TonB-dependent receptor | 546         | +5.41  | +7.88  | 18.0(13)       | -177  |
| exbD            | b3005       | Fe-siderophore & B12 transport | 57          | +3.93  | +6.35  |                |       |
| exbB            | b3006       | Fe-siderophore & B12 transport | 229         | +3.17  | +4.84  |                |       |
| ygiH            | b3070       | like siderophore-utilization gene viuB | 85          | +2.17  | +1.72  | 13.5(14)       | -47   |
| bfd             | b3337       | release of Fe from Bfr? | 58          | +4.40  | +7.53  | 17.5(11)       | -34   |
| feoA            | b3408       | Fe(II) transport | 70          | +1.66  | +3.65  | 14.3(13)       | -114  |
| feoB            | b3409       | Fe(II) transport | 173         | +2.35  | +2.34  |                |       |
| fecE            | b4287       | Fe-citrate transport | 69          | +2.40  | +3.32  |                |       |
| fecD            | b4288       | Fe-citrate transport | 172         | +1.12  | +2.65  |                |       |
| Genes   | Description                                | Gene ID | Expression Level |
|---------|--------------------------------------------|---------|-----------------|
| fecC    | Fe-citrate transport                       | b4289   | 56, +3.03, +10.76 |
| fecB    | Fe-citrate transport                       | b4290   | 76, +7.15, +27.31 |
| fecA    | Fe-citrate transport                       | b4291   | 1,482, +3.69, +6.72, 18.8(12), -67 |
| fecR    | Fe-citrate transport regulator              | b4292   | 23, +5.56, +14.22 |
| fecI    | Fe-citrate transport regulator              | b4293   | 14, +8.28, +18.12, 19.6(15), -45 |
| fhuF    | Reduction of Fe(III) in ferrioxamine B      | b4367   | 92, +7.24, +11.34, 20.7(17), -104 |

1Potential novel iron transporters are boxed, gene orientation is indicated by left (clockwise) and right (counter clockwise) justification, and transcriptional organization is indicated by the vertical arrows. 2Relative expression levels for the wild type in L broth as parts per million (ppm). 3Fold increases (+) and decreases (-) in expression levels (with respect to wild type in L broth) caused by dip or the fur mutation are indicated by black and white backgrounds, respectively. 4Expression changes of less than twofold are shown with gray backgrounds and were included either because the corresponding genes are associated with adjacent Fe²⁺-Fur regulated genes or because the expression changes are considered significant (<0.05 probability in Student’s t-test). 5The data for the genes of the fhuACDB operon are combined and are included for completion sake although they do not show any major change in expression. 6The most strongly predicted Fur-binding site is indicated with the probability score (and number of base-pairs matching the 19 bp consensus indicated in brackets) determined as described by Robison et al. (18) using 19 known Fur sites with an average probability score of 19.6 (high scoring sites are more strongly predicted than low scoring sites). ‘NI’ indicates that no Fur-binding site was predicted using the above approach. 7The distance of the Fur site upstream (negative) or downstream (positive) of the translational start site of the corresponding gene is indicated. 8Descriptions are mostly derived from ExPASy.
| Gene       | Blattner No. | Description                  | Level (ppm) | Dip  | fur  | Fe   | Fur site | Score | Pos. |
|------------|--------------|-------------------------------|-------------|------|------|------|----------|-------|------|
| cyoA-E     | b428-32      | cytochrome bo oxidase         | 587         | -1.54 | -1.56 | 3    | 21.6(13) | -249  |      |
| glutA      | b0720        | citrate synthase              | 116         | +2.31 | +2.04 | NI   |          |       |      |
| cydA       | b0733        | cytochrome bd oxidase 1 (heme b<sub>558</sub>+593) | 104         | -1.67 | -3.01 | 2    | NI       |       |      |
| cydB       | b0734        | cytochrome bd oxidase 1 (heme d) | 182         | -2.45 | -3.68 | 1    |          |       |      |
| ybgE       | b0735        | cytochrome bd oxidase 1 related? | 25          | -6.09 | -9.88 |     |          |       |      |
| gpmA       | b0755        | phosphoglycerate mutase I     | 5           | +1.89 | +3.61 |      |          |       |      |
| appC       | b0978        | cytochrome bd oxidase 2, s-u I (heme b) | 15          | -1.78 | -1.60 | 2    | NI       |       |      |
| appB       | b0979        | cytochrome bd oxidase 2, s-u II (heme d) | 14          | -2.37 | -2.40 | 1    |          |       |      |
| narK       | b1223        | nitrite exporter 1            | 780         | -3.81 | -8.66 | 43   | NI       |       |      |
| narG       | b1224        | resp. nitrate red-ase 1 α s-u; Mo, 4Fe-4S | 79          | -6.73 | -4.07 | 4    |          |       |      |
| narH       | b1225        | resp. nitrate red-ase 1 β s-u; 4x 3/4Fe-4S | 30          | -7.09 | -4.07 | 16   |          |       |      |
| narJ       | b1226        | resp. nitrate red-ase 1 δ s-u | 16          | -2.38 | 2.03  | 1    |          |       |      |
| narI       | b1227        | resp. nitrate red-ase 1 γ s-u; 2x heme b<sub>556</sub> | 16          | -1.70 | -1.49 | 2    |          |       |      |
| paaY       | b1400        | phenylacetate degradation?    | 4           | -2.14 | -2.06 | 1    |          |       |      |
| ydeE       | b1464        | Oxidoredox-ase?               | 16          | +1.03 | -1.02 | 1    |          |       |      |
| narV       | b1465        | resp. nitrate red-ase 2 γ s-u; heme b | 4           | +1.14 | -1.24 | 1    |          |       |      |
| narW       | b1466        | resp. nitrate red-ase 2 δ s-u | 1           | -1.95 | -1.31 | 1    |          |       |      |
| narγ       | b1467        | resp. nitrate red-ase 2 β s-u; 4x 3/4Fe-4S | 6           | -2.35 | -2.11 | 16   |          |       |      |
| narZ       | b1468        | resp. nitrate red-ase 2 α s-u; Mo, 4Fe-4S | 12          | -3.91 | -13.25 | 4   |          |       |      |
| narU       | b1469        | nitrite exporter 2            | 1           | +1.37 | +1.61 | 1    |          |       |      |
| ccmA       | b2201        | cytochrome maturation; heme exporter | 7           | -1.98 | -1.74 |     |          |       |      |
| napC       | b2202        | periplasmic nitrate red-ase; 4x heme | 6           | -3.26 | -2.17 | 4    |          |       |      |
| napB       | b2203        | periplasmic nitrate red-ase; 2x heme | 5           | -2.27 | -2.62 | 2    |          |       |      |
| napH       | b2204        | periplasmic nitrate red-ase; 4x 4Fe-4S | 7           | -1.40 | -1.64 | 8    |          |       |      |
| napG       | b2205        | periplasmic nitrate red-ase; 4x 4Fe-4S | 53          | -1.66 | -1.30 | 16   |          |       |      |
| napA       | b2206        | periplasmic nitrate red-ase; Mo, 4Fe-4S | 19          | -7.02 | -6.40 | 4    |          |       |      |
| napD       | b2207        | assembly of Nap complex?      | 111         | -1.46 | -1.39 | 1    |          |       |      |
| napE       | b2208        | periplasmic nitrate red-ase; 3x 4Fe-4S | 11          | -4.25 | -3.49 | 12   | 14.6(12) | -193  |      |
| tdcG-A     | b3112-8      | anaerobic Thr dehydratase; 4Fe-4S? | 292         | -1.67 | -2.95 | 4    |          |       |      |
| garK       | b3124        | glycerate kinase 2            | 24          | -2.41 | -2.03 | 1    |          |       |      |
| garR       | b3125        | red-ase; galactarate metabolism | 21          | -1.80 | -1.09 | 1    |          |       |      |
| garL       | b3126        | 2-dehydro-3-deoxyglucarate aldolase | 57          | -1.64 | -3.87 | 1    |          |       |      |
| garP       | b3127        | galactarate transporter       | 21          | -2.09 | -3.38 | 1    |          |       |      |
| nirB       | b3365        | NAD(P)H-nitrite red-ase; siroheme, 2 Fe-S | 158         | -1.42 | 1.52  | 9    |          |       |      |
| nirD       | b3366        | NAD(P)H-nitrite red-ase small subunit | 11          | -2.19 | -2.83 | 1    |          |       |      |
| nirC       | b3367        | nitrite transporter?          | 12          | -2.68 | -2.22 | 1    |          |       |      |
| gntU       | b3436        | gluconate transporter         | 5           | +1.22 | +1.19 | 1    |          |       |      |
| gntK       | b3437        | catabolic glucokinase 2       | 22          | +2.50 | +2.44 | 1    |          |       |      |
| gntR       | b3438        | gnt operon transcriptional repressor | 12          | +1.79 | +1.79 | 1    |          |       |      |
| frdD       | b4151        | fumarate red-ase; anchor polypeptide | 9           | -3.83 | -4.41 | 1    |          |       |      |
| frdC       | b4152        | fumarate red-ase; anchor polypeptide | 7           | -4.15 | -5.46 | 1    |          |       |      |
| frdB       | b4153        | fumarate red-ase; 2Fe-2S, 3Fe-4S,4Fe-4S | 20          | -2.59 | -5.06 | 9    |          |       |      |
| frdA       | b4154        | fumarate red-ase; flavoprotein | 39          | -1.49 | -1.76 | 1    |          |       |      |

Total Fe atoms/subunit: 148
The data for the genes of the *cyo, nuo* and *tdc* operons are combined to reduce the Table size. Abbreviations: s-u, subunit; and red-ase, reductase. The number of Fe atoms per encoded protein is indicated. All other details are as for Table I.
**Table 3.**

Quantitative RT-PCR analysis of iron and Fur-dependent gene regulation

| Gene  | Macroarray Dip | fur | RT-PCR Dip | fur |
|-------|----------------|-----|------------|-----|
| entA  | +9.60          | +17.3 | +133 | +43.0 |
| ybiL  | +30.7          | +38.0 | +20.0 | +50.9 |
| ycdB  | +2.60          | +2.48 | +17.3 | +22.2 |
| pqQL  | +4.29          | +2.31 | +28.6 | +26.2 |
| ydiE  | +2.91          | +8.12 | +28.2 | +18.6 |
| narH  | -7.09          | -4.07 | -3.7 | -2.2 |
| narZ  | -3.91          | -13.25 | -2.0 | -2.1 |
| napA  | -7.02          | -6.40 | -123 | -19.4 |
| nuoE  | -1.99          | -2.60 | -3.30 | -4.30 |
| nuoI  | -1.70          | -2.42 | -2.80 | -2.90 |
| frdB  | -2.59          | -5.06 | -7.1 | -3.1 |
| sodB  | -8.30          | -12.45 | -126 | -72.5 |
| polA  | -1.04          | +1.02 | +1.66 | +1.30 |

Fold changes in expression with respect to the wild type in L broth were determined using 1macroarrays and 2quantitative RT-PCR. Other details are as for Table I.

3Included as positive controls, 4included as a negative control.
### Miscellaneous iron- and Fur-regulated genes.

| Gene       | Blattner No. | Description                                                                 | Level (ppm) | Dip     | fur     | Fur site |
|------------|--------------|------------------------------------------------------------------------------|-------------|---------|---------|----------|
| **Metal metabolism**                            |              |                                                                              |             |         |         |          |
| nikA       | b3476        | Ni transport (for Ni-hydrogenases)                                           | 1372        | -1.95   | +1.11   | NI       |
| nikB       | b3477        | Ni transport (for Ni-hydrogenases)                                           | 717         | -1.67   | 1.00    |          |
| nikC       | b3478        | Ni transport (for Ni-hydrogenases)                                           | 432         | -2.50   | -1.43   |          |
| nikD       | b3479        | Ni transport (for Ni-hydrogenases)                                           | 435         | -4.76   | -2.51   |          |
| nikE       | b3480        | Ni transport (for Ni-hydrogenases)                                           | 395         | -1.84   | -1.33   |          |
| nikR       | b3481        | Ni-dependent nik-operon regulator                                            | 665         | -2.05   | -2.00   |          |
| ymfD       | b1137        | methyl transferase? tellurite resistance?                                    | 53          | -2.44   | -2.16   | NI       |
| ymfE       | b1138        | integral-membrane protein - probable                                         | 591         | -1.88   | -1.71   |          |
| **Phage related**                               |              |                                                                              |             |         |         |          |
| ybcC       | b0539        | like exo of phage λ                                                         | 81          | +2.13   | +2.17   | NI       |
| ynaC       | b1375        | like ydf/K; Rac prophage gene                                                | 7682        | -5.51   | -4.17   | 18.8(15) -47 |
| ydfK       | b1544        | like ydfE; Qin prophage gene                                                 | 5227        | -5.44   | -3.94   | NI       |
| cspF       | b1557        | cold shock; Qin prophage gene                                                | 555         | -3.45   | -2.18   |          |
| cspB       | b1557        | cold shock; Qin prophage gene                                                | 4529        | -2.92   | -3.41   | NI       |
| **Stress**                                        |              |                                                                              |             |         |         |          |
| sodB       | b1656        | Fe superoxide dismutase                                                      | 5439        | -8.30   | -12.45  | NI       |
| yldE       | b3685        | integral membrane protein                                                    | 1318        | +1.85   | +1.45   |          |
| hslS       | b3686        | ibpB; small heat shock family                                                | 80          | +6.67   | +3.00   |          |
| hslT       | b3687        | ibpA; small heat shock family                                                | 135         | +6.67   | +2.63   | NI       |
| **Nucleic acid/nucleotide metabolism**           |              |                                                                              |             |         |         |          |
| codB       | b0336        | cytosine permease                                                           | 147         | +5.56   | +4.54   | NI       |
| nrdH       | b2673        | ribonuc. red-ase 2? glutaredoxin-like                                        | 242         | +1.79   | +2.00   | 16.9(13) -226 |
| nrdI       | b2674        | ribonuc. red-ase 2? unknown role                                             | 158         | +2.22   | +4.54   |          |
| nrdE       | b2675        | ribonuc. red-ase 2; α chain, catalytic                                       | 1275        | +1.67   | +2.5    |          |
| nrdF       | b2676        | ribonuc. red-ase 2; β chain, 2 Fe atoms                                      | 283         | +2.44   | +3.6    |          |
| ygQ        | b2884        | xanthine/uracil permease?                                                    | 78          | +4.00   | +2.63   | NI       |
| rtcA       | b3619        | RNA cyclase; Mg/Mn cofactor                                                 | 256         | +2.94   | +2.17   | NI       |
| pyrL       | b4246        | leader peptide; Asp transcarbamoylase                                        | 125         | -2.15   | -2.38   | NI       |
| **Various/Unknown**                             |              |                                                                              |             |         |         |          |
| ycaN       | b0900        | LysR-like transcriptional regulator                                          | 3479        | +4.35   | +5.26   | NI       |
| ompW       | b1256        | outer-membrane protein                                                       | 1244        | -4.76   | -3.59   | NI       |
| ydhW       | b1672        | unknown                                                                      | 118         | +2.56   | +2.33   | NI       |
| yoaD       | b1815        | diguanylate cyclase/phosphodiesterase?                                      | 1384        | +2.88   | +2.22   | NI       |
| cheZ       | b1881        | regulation of flagellar rotation                                            | 3581        | -3.43   | -4.84   | NI       |
| yeeL/R     | b1979        | transfer heptose to lipopolysaccharide?                                      | 84          | -2.41   | -3.32   |          |
| yeeO       | b1985        | multi-antimicrobial extrusion family                                        | 14964       | -2.20   | -2.38   | NI       |
| yeeR       | b2001        | flagellin related?                                                           | 8153        | -2.38   | -2.77   | NI       |
| yfaH       | b2238        | unknown                                                                      | 75          | -2.65   | -2.71   | NI       |
| cinA/yfaA  | b2249        | like Mo-cofactor biosyn protein                                              | 4102        | -2.08   | -2.14   |          |
| elaC       | b2268        | like arylsulfatase & diZn-β-lactamase                                       | 1925        | -2.38   | -2.11   | NI       |
| yfdI       | b2579        | like Pf - contains Gly radical region                                        | 7341        | -3.23   | -2.58   | NI       |
| ygeQ       | b2863        | unknown                                                                      | 85          | +4.35   | +2.7    | NI       |
| yggT       | b2959        | unknown                                                                      | 1279        | -2.18   | -2.14   | NI       |
| rplP       | b3313        | 50S ribosomal subunit protein L16                                            | 10588       | -2.20   | -2.06   | NI       |
| rplW       | b3318        | 50S ribosomal subunit protein L23                                            | 4120        | -3.00   | -2.18   | NI       |
| yhgI       | b3414        | unknown                                                                      | 506         | +2.44   | +2.50   | NI       |
| yhhY       | b3441        | similar to the acetyltransferase family                                     | 244         | -2.36   | -2.53   | 19.9(16) -214 |
| Gene       | Function                          | Location | Log2 Fold Change | t-value | p-value | Class |
|------------|-----------------------------------|----------|-----------------|---------|---------|-------|
| phnT       | in phosphonate/C-P lyase operon   | 811      | -4.01           | -11.31  | NI      |       |
| fimE       | fimbriae regulation; recombinase  | 41       | +6.67           | +3.23   | NI      |       |

1 Likely to be associated with *yhhX (ryhB)* rather than *yhhY* (11). Other details as for Table I.
FIG. 1. **Effect of the fur mutation on the iron-protein composition of *E. coli***.

Autoradiograph of $^{55}$Fe-labeled *E. coli* soluble cell extracts (0.15 OD$_{650}$nm units per lane) fractionated by 6% native-PAGE with 0.1% Triton X-100. MC4100 (‘wt’) and H1941 (‘fur’) were grown aerobically in L broth to stationary phase. The position of the band corresponding to Bfr is indicated (identified by analysis of *bfr* mutant strains; data not shown).

FIG. 2. **Effect of 2,2’-dipyridyl and the fur mutation on the cytochrome composition of *E. coli***. Cytochrome composition was determined by room-temperature reduced minus oxidized difference spectroscopy of whole cells harvested in the late log phase (OD$_{650}$nm 1.0) following aerobic growth in L broth ± 200 µM dip. Spectra for the wild type (MC4100; ‘wt’), the wild type grown with dip (‘wt + dip’) and the *fur* mutant (H1941; ‘fur’) are shown, and the cytochrome *d* signal at 630 nm is indicated as is a signal at 560 nm derived from several *b*-type cytochromes. Note that spectra taken from cells grown to OD$_{650}$nm 0.5 and 3.0 showed similar dip and *fur*-induced effects on cytochrome *b* and *d* content (not shown).

FIG. 3. **Effect of the fur mutation on the Fe-S cluster composition of *E. coli***. Fe-S cluster composition was determined by EPR spectroscopy in the as prepared (oxidized) and dithionite-reduced whole cells harvested in the mid-exponential phase (OD$_{650}$nm 0.5) following aerobic growth in L broth. Spectra for MC4100 (‘wt’) and H1941 (‘fur’) are shown. A. The position of the components of signals from the 3Fe-4S clusters (g=2.019, paramagnetic in the oxidized state) and signals from the centers spectrally identical to those in complex I (N1b, $g_1=2.022$, $g_2=1.935$, $g_3=1.924$; N2, $g_1=2.052$, $g_2=g_3=1.936$; N3, $g_1=2.03$, $g_2=1.93$, $g_3=1.87$; N5, $g_1=2.078$, $g_2=1.93$, $g_3=1.90$; all paramagnetic in the reduced state (35, 38)) are indicated. The prominent
feature at $g \sim 1.935$ (arrowed) results from a superimposition of signals from 4Fe-4S clusters. Note: the wt reduced spectrum was recorded at half amplification with respect to the other spectra. **B.** The same spectra recorded at a lower field range; the signal from the mononuclear non-heme iron centers ($g=4.3$) is indicated. Broadly similar results were obtained for cells grown to OD$_{650nm}$ 1.0 and 3.0 (not shown). Spectra with dip are not shown due to interference from a large Mn$^{2+}$ signal, presumably arising from an Mn$^{2+}$-dip complex.
REFERENCES

1. Andrews, S.C. (1998) *Adv. Microb. Physiol.* **40**, 281-351

2. Guerinot, M-L. (1994) *Annu. Rev. Microbiol.* **48**, 743-772

3. Earhart, C.F. (1996) In ‘*Escherichia coli* and *Salmonella*: Cellular & Molecular Biology’, pp 1075-1090, Ed F.C. Neidhardt, 2nd Ed, ASM Press, Washington

4. Hantke, K. (2001) *Curr. Opin. Microbiol.* **4**, 172-177

5. Abdul-Tehrani, H., Hudson, A. J., Chang, Y-S., Timms, A. R., Hawkins, C., Williams, J. M., Harrison, P. M, Guest J. R. & Andrews, S. C. (1999) *J. Bacteriol.* **181**, 1415-1428

6. Stojilkovic, I., Bäumler, A.J. & Hantke, K. (1994) *J. Mol. Biol.* **236**, 531-545

7. Park, S.J. & Gunsalus, R.P. (1995) *J. Bacteriol.* **177**, 6255-6262

8. Vassinoiva, N. & Kozyruv, D. (2000) *Microbiol.* **146**, 3171-3182

9. Touati, D. (1988) *J. Bacteriol.* **170**, 2511-2520

10. Tseng, C-P. (1997) *FEMS Microbiol. Lett.* **157**, 67-72

11. Massé, E. & Gottesman, S. (2002). *Proc. Natl. Acad. Sci. USA* **99**, 4620-4625

12. Sambrook, J., Fritsch, E.F. & Maniatis, T. (1989) In ‘Molecular Cloning: A Laboratory Manual’, 2nd Ed. Cold Spring Harbor Laboratory Press, NY.

13. Kalnenieks, U., Galinina, N., Bringer-Meyer, S. & Poole, R. K. (1998). *FEMS Microbiol. Lett.* **168**, 91-97.

14. Jones, C.W. & Redfearn, E.R. (1966) *Biochim. Biophys. Acta* **113**, 467-481

15. Kita, K., Konishi, K. & Anraku, Y. (1984) *J. Biol. Chem.* **259**, 3369-3374

16. Markwell, M.A.K., Haas, S. M., Bieber, L. L.& Tolbert, N. E (1978) *Anal. Biochem.* **87**, 206-210

17. Philips-Jones, M.K., Watson, F.J. & Martin, R. (1993) *J. Mol. Biol.* **233**, 1-6

18. Robison, K., McGuire, A.M. & Church, G.M. (1998) *J. Mol. Biol.* **284**, 241-254
19. Hantke, K. (1981) *Mol. Gen. Genet.* **182**, 288-292
20. Zheng, M., Wang, X., Templeton, L.J., Smulski, D.R., LaRossa, R.A. & Storz, G. (2001) *J. Bacteriol.* **183**, 4562-4570
21. Patzer, S.I. & Hantke, K. (1999) *J. Bacteriol.* **181**, 3307-3309
22. Quail, M.A., Jordan, P., Grogan, J.M., Butt, J.N., Lutz, M., Thomson, A.J., Andrews, S.C. & Guest, J.R. (1996) *Biochem. Biophys. Res. Commun.* **229**, 635-642
23. Hantke, K. (1990) *FEMS Microbiol. Lett.* **67**, 5-8
24. Ochsner, U.A. & Vasil, M.L. (1996) *Proc. Natl. Acad. Sci. USA* **93**, 4409-4414
25. Stanley, N.R., Findlay, K., Berks, B.C. & Palmer, T. (2001) *J. Bacteriol.* **183**, 139-144
26. Stearman, R., Yuan, D.S., Yamaguchi-Iwai, Y., Klausner, R.D. & Dancis, A. (1996) *Science* **271**, 1552-1557
27. Stojiljkovic, I. & Hantke, K. (1992) *EMBO J.* **11**, 4359-4367
28. Tsolis, R.M., Baumler, A.J., Stojiljkovic, I. & Heffron, F. (1995) *J. Bacteriol.* **177**, 4628-4637
29. Panina, E.M., Mironov, A.A. & Gelfand, M.S. (2001) *Nucleic Acids Res.* **29**, 5195-5206
30. Cotter, P.A., Darie, S. & Gunsalus, R.P. (1992) *FEMS Microbiol. Lett.* **100**, 227-232
31. Spiro, S. & Guest, J.R. (1990) *FEMS Microbiol Rev.* **75**, 399-428
32. Rainnie, D.J. & Bragg, P.D. (1973) *J. Gen. Microbiol.* **77**, 339-349
33. Bolgiano, B., Salmon, I., Ingeldew, W.J. & Poole, R.K. (1991) *Biochem. J.* **274**, 723-730
34. Gennis, R.B. & Stewart, V. (1996) In ‘Escherichia coli and Salmonella: Cellular & Molecular Biology’, pp 217-261, Ed F.C Neidhardt, 2nd Ed, ASM Press, Washington

35. Cammack, R. & Cooper, C. E. (1992) Meth. Enzymol. 227, 353-384

36. Park, S-J, Tseng, C-P. & Gunsalus, R.P. (1995) Mol. Microbiol. 15, 473-482

37. Keyer, K. & Imlay, J.A. (1996) Proc. Natl. Acad. Sci. USA 93, 13635-13640

38. Ohnishi, T. (1998) Biochim. Biophys. Acta 1364, 186-206

39. Niederhoffer, E.C., Naranjo, C.M., Bradley, K.L. & Fee, J.A. (1990) J. Bacteriol. 172, 1930-1938

40. Eitinger, T. & Mandrand-Berthelot, M.A. (2000) Arch. Microbiol. 173, 1-9

41. Jordan, A., Aragall, E., Gibert, I. & Barbe, J. (1996) Mol. Microbiol. 19, 777-790

42. Gruer, M.J. & Guest, J.R. (1994) Microbiol. 140, 2531-2541

43. Baichoo, N., Wang, T., Ye, R. and Helmann, J.D. (2002) Mol. Microbiol. 45, 1613-1629

44. Thompson, D.K., Beliaev, A.S., Giometti, C.S., Tollaksen, S.L., Khare, T., Lies, D.P., Nealson, K.H., Lim, H., Yates III, J., Brandt, C.C., Tiedje, J.M. and Zhou, J. (2002) Appl. Environ. Microbiol. 68, 881-892

45. Paustian, M.L., May, B.J. and Kapur, V. (2001) Infect. Immun. 69, 4109-4115

46. Ochsner, U.A., Wilderman, P.J., Vasil, A.I. and Vasil, M.L. (2002). Mol. Microbiol. 45, 1277-1287
Fig. 1

wt  fur

Bfr →
Fig. 2

\[ \Delta A = 0.005 \]

- **wt**
- **wt + dip**
- **fur**

**b-type cytochromes**

**cytochrome d**

Wavelength (nm)
Fig. 3

A

$g = 1.935$

$3\text{Fe}-4\text{S}$

B

$g = 4.3$
Fig. 3

A

$g = 1.935$

fur oxidized

wt oxidized

fur reduced

wt reduced

$\times 0.5$

Magnetic field, Gauss

3060 3160 3260 3360 3460 3560 3660

B

$g = 4.3$

fur oxidized

wt oxidized

fur reduced

wt reduced

Magnetic field, Gauss

1360 1460 1560 1660 1760 1860 1960
Global iron-dependent gene regulation in Escherichia coli: A new mechanism for iron homeostasis

Jonathan P. McHugh, Francisco Rodriguez-Quinones, Hossein Abdul-Tehrani, Dimitri A. Svistunenko, Robert K. Poole, Chris E. Cooper and Simon C. Andrews

J. Biol. Chem. published online May 13, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M303381200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts