Escherichia coli Transcriptome Dynamics during the Transition from Anaerobic to Aerobic Conditions

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Escherichia coli is a metabolically versatile bacterium that is able to grow in the presence and absence of oxygen. As an inhabitant of the lower intestine, E. coli often occupies an oxygen-starved niche only to be excreted from the host into an aerobic external environment. The ability to adapt to such changes is rooted in altered patterns of gene expression in response to key cues, such as O2 availability.

Depending on the availabilities of electron donors and acceptors, E. coli uses one of three metabolic modes to support growth (1). In the presence of O2, aerobic respiration allows the complete oxidation of a growth substrate and therefore is the most productive and hence the preferred metabolic mode (1). During aerobic metabolism, the glycolytic conversion of glucose to pyruvate and the oxidative decarboxylation of pyruvate by the pyruvate dehydrogenase complex leads to the formation of acetyl-CoA, which feeds the citric acid cycle (CAC). Oxidation of the acetyl units generates reducing equivalents that are transferred to O2 via aerobic electron transport chains, creating proton gradients that can be used to generate ATP and drive other essential activities (2). In the absence of O2, two alternative metabolic modes are available. If a terminal electron acceptor, such as NO3−, is present, then anaerobic respiration is possible. During anaerobic respiration the role of the pyruvate dehydrogenase complex is assumed by pyruvate formate-lyase; the CAC is repressed, and the metabolic flow in the C4 section of the CAC is reversed. Anaerobic respiration yields less energy than aerobic respiration because the substrate is only partially oxidized (1). However, anaerobic respiration is more productive than fermentation, where energy is conserved by substrate level phosphorylation, and redox balance is achieved by the formation of acetate, ethanol, H2, CO2, formate, succinate, and lactate (3). Thus, enormous changes in E. coli physiology are provoked by changes in O2 availability.

Previous reports of the effects of O2 on the transcript profile of E. coli have compared separate aerobic and anaerobic cultures, usually grown as batch cultures (4–7). Consequently, there is little information on transcriptome dynamics during adaptation to aerobic growth. Here, the transcriptional responses of steady-state anaerobic chemostat cultures upon exposure to air are reported. Within 5 min the abundances of transcripts associated with anaerobic metabolism were decreased, whereas transcripts associated with aerobic metabolism and the peroxide stress response were increased. Further adaptations include transient expression of transcripts encoding methionine biosynthesis and putrescine degradation proteins that are revealed as previously unrecognized pathways in successful adaptation to aerobic conditions.

**EXPERIMENTAL PROCEDURES**

**Bacterial Strains and Growth Conditions**—The strains of E. coli and the plasmids used in this study are listed in supplemental Table 1. E. coli strain MG1655 was used for the transcript profiling experiments. Chemostat cultures were grown in New Brunswick Scientific Bioflow 1000 fermentation vessels (1.8-liter capacity) with the culture agitation speed set at 400 rpm and the temperature maintained at 37 °C. Oxygen levels were monitored using galvanic oxygen electrodes, and the pH was maintained at 7.2 ± 0.2 by automatic titration with sterile KOH. Carbon-limited Evans defined medium (8) was used as the growth medium with glucose (30 mM) as the carbon source. Inocula were grown overnight as batch cultures in the defined culture medium, whereas steady-state cultures were verified through measurement of culture density over several generations with the dilution rate being 0.2 h−1. Anaerobic cultures were maintained by sparging the chemostat with oxygen-free nitrogen (95%) and carbon dioxide (5%) (0.2 liters min−1). The switch to aerobic conditions was achieved by sparging the culture with air (2.0 liters min−1) in place of the above gas mixture. Anaerobic over-metabolites (lactate, succinate, ethanol, and acetate) in culture supernatants and residual glucose concen-
trations were measured using kits obtained from Roche Applied Science. Formate was measured by the method of Lang and Lang (9). The amounts of NADH and NAD\(^+\) were determined essentially as described by Benofsky and Swan (10).

**E. coli** MG1655, isogenic metR and puuA strains, and MC4100 and MC4100 oxyR were used to investigate the effects of the indicated mutations on the anaerobic- aerobic transition. Cultures were grown anaerobically in sealed bottles in glucose-supplemented Evans medium (8) at 37 °C to \(A_{630} 0.2–0.3\). Approximately equal numbers of the parent and mutant were mixed under anaerobic conditions (Don Whitley Mk3 Anaerobic Work station), and then incubated for a further 2 h under either aerobic or anaerobic conditions before determining the number of colony-forming units on L agar and L agar containing kanamycin (30 mg l\(^{-1}\)). Competitive index (CI) values were calculated from the colony-forming units as follows: mutant\(_{\text{aerobic}}/\text{parent}\text{aerobic}\) mutant\(_{\text{anaerobic}}/\text{parent}\text{anaerobic}\). For the metR mutant the medium was supplemented with methionine (1 \(\mu\)g/l) to allow growth. For chemical complementation studies, methionine (1 mM) or putrescine (20 mM) was added as indicated.

**Transcript Profiling**—Culture samples (10 ml; an insignificant amount of the total culture volume of 1.8 liters) were flushed from the chemostat directly into RNA Protect (Qiagen) to stabilize the RNA before purification using a RNAsy Midi kit (Qiagen) and on column DNase treatment following the manufacturer’s instructions. The integrity of the RNA samples was determined by gel electrophoresis, and concentration and purity were determined using an Eppendorf BioPhotometer. Equal quantities of RNA from control (anaerobic steady state) and experimental samples (5–10, 15–, and 60-min exposure to air) were labeled with Cy3-dCTP or Cy5-dCTP fluorescent dyes. For each **E. coli** K12 OciChip (Ocimum Biosolutions, product number 2140–000000, 50-mer oligo array), one sample was labeled with Cy3, and another was labeled with Cy5. Dye swap experiments for each pairing, with two biological replicates (separate chemostat runs), were used to compensate for any incorporation differences between the two Cy dyes. Synthesis of cDNA and subsequent hybridization and washing steps were as described by Flatley et al. (12).

Data analysis was carried out using Imagene, version 5.1, and Genesight, version 4 (Biodiscovery Inc). The mean values from each channel were \(\log_2\)-transformed and normalized using the LOWESS algorithm to remove intensity-dependent effects within the calculated values. Normalized values were used to calculate the Cy3:Cy5 fluorescence ratios from experimental and biological repeats with all replicates combined. Data were compiled from two aerobic and two anaerobic cultures, each with a dye-swap replicate, thus providing two biological repeats within four technical repeats. Genes showing greater than 2-fold change in transcript abundance at one or more of the four time points with a \(p\) value of \(<0.05\) (as determined by a \(t\) test) were deemed to be differentially regulated.

**Real Time PCR**—RNA was extracted as described above. cDNA was synthesized from 4 \(\mu\)g of starting material, primed with 9 \(\mu\)g of pd(N)6 random hexamers (Amersham Biosciences). Reaction mixtures (20 \(\mu\)l) containing 0.5 mM dATP, dTTP, dGTP, and dCTP were incubated for 2 h at 42 °C with 200 units of Superscript II RNase-H reverse transcriptase (Invitrogen). The resulting cDNA was purified using a PCR purification kit (Qiagen) to remove unincorporated dNTPs and primers. Gene-specific primers were designed to amplify 50–150 nucleotide fragments of target genes using PRIMER 3 software (13). Reactions (25 \(\mu\)l) contained 12.5 \(\mu\)l of 2× Sensimix Quanitate buffer, 0.5 \(\mu\)l of 50× SYBR\(^\text{®}\) green solution (QuantaTide Ltd., London, UK), 5 pmol of each of the two primers, and 5 \(\mu\)l of cDNA sample in a 96-well optical reaction plate mounted in an ABI 7700 thermocycler (Applied Biosystems). The thermal cycling conditions were as follows: 50 °C for 2 min; 95 °C for 10 min; 40 cycles of 95 °C for 15 s; 60 °C for 1 min. A standard curve was established using genomic DNA for each gene studied to confirm that the primers amplified at the same rate and to validate the experiment. The relative levels of expression of genes of interest compared with untreated controls were calculated following the protocol for the Standard Curve Method in the User Bulletin 2 (ABI Prism 7700 Sequence Detection System, Relative Quantification of Gene Expression) supplied by Applied Biosystems. No template reactions were included as negative controls.

**YacL–AcnB Interactions**—To investigate AcnB-YacL interactions in vivo, the **E. coli** cya mutant (BTH101), which is adenylate cyclase-deficient, was transformed with appropriate combinations of the pKT25 and pUT18C derivatives. The pKT25 and pUT18C plasmids allow the creation of in-frame fusions of the T25 and T18 catalytic domains of Bordetella pertussis adenylate cyclase (14) with AcnB and YacL. Standard methods were used in plasmid construction (11). The DNA inserts were amplified from **E. coli** genomic DNA by PCR and ligated between the PstI and BamHI sites of plasmids pKT25 and pUT18C. In addition, pKT25 derivatives containing AcnB domains 1–2–3 (amino acids 370–865) and AcnB domains 5–4 (amino acids 1–382) were created, for use in combination with a pUT18C-YacL plasmid (supplemental Table 1). Cultures containing the indicated plasmids were grown in L broth (15) containing ampicillin (100 \(\mu\)g ml\(^{-1}\)), kanamycin (50 \(\mu\)g ml\(^{-1}\)), and chloramphenicol (30 \(\mu\)g ml\(^{-1}\)).

Determinations of DNA Supercoiling—**E. coli** MG1655 and the isogenic **puuA** mutant were transformed with plasmid pGEM3Zf(+) (Promega). Anaerobic cultures were grown at 37 °C in Evan’s medium (8) supplemented with glucose (30 mM) and ampicillin (200 mg liter\(^{-1}\)). Aliquots were removed for isolation of plasmid DNA using Qiagen spin kits (Qiagen), and then the cultures were aerated. Further samples were taken 5, 10, 15, and 60 min after culture aeration. Plasmid DNA was analyzed by Tris borate-buffered agarose (1%) gel electrophoresis (11) in the presence of 25 \(\mu\)g ml\(^{-1}\) chloroquine essentially as described by Dorman et al. (17). Under these conditions the least supercoiled topoisomers migrate furthest in the gels.

**RESULTS AND DISCUSSION**

**Global Transcriptional Responses upon Exposure of Anaerobic Chemostat Cultures to Air**—Combining chemostats with transcript profiling provides a sound experimental basis for investigating the transcriptional response of **E. coli** during the
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transition between anaerobic and aerobic growth. Steady-state cultures provide reproducible starting points for studying the dynamics of the transcriptome (18). Specifically, the growth rate, medium composition, pH value, and aeration of the culture were tightly controlled. Therefore, glucose-limited chemostat cultures were established, in which no O2 could be detected polarographically throughout growth. Rates of production of fermentation products were (mmol h^-1 g dry weight^-1) as follows: acetate, 6.1 ± 0.3; ethanol, 2.8 ± 0.1; succinate, 1.6 ± 0.2; formate 1.1 ± 0.1; and lactate 0.6 ± 0.03, and there was no detectable glucose in the culture outflow. These observations are consistent with E. coli growing by glucose fermentation (19). Samples from the anaerobic steady-state cultures were rapidly flushed into RNA protect (Qiagen) for isolation of total RNA. The system was then perturbed by the introduction of air. Samples were obtained for RNA preparation 5, 10, 15, and 60 min after the introduction of air. The corresponding labeled cDNAs were synthesized and used to probe E. coli K12 arrays (Ocimum Biosolutions). A second chemostat run for the anaerobic-aerobic transition was set up, and in each case the Cy-dye pairings were reversed to provide two biological replicates and two technical replicates.

Many decades of intensive investigation of E. coli physiology and gene expression under both aerobic and anaerobic conditions provide a large number of transcripts that should respond in a predictable manner to validate the array data. A significant change in transcript abundance was defined as at least 2-fold (p = 0.05) at one or more of the time points analyzed. According to these criteria 278 individual gene transcripts were more abundant, and 259 gene transcripts were less abundant, at one or more time point compared with the anaerobic starting state. The full data set is provided as supplemental material (supplemental Table 2; GEO accession number GSE4735).

CAC and Aerobic Respiratory Chain Transcripts Exhibit Rapid Responses to the Presence of Oxygen—During the 5 min following the introduction of air, most CAC transcripts increase in abundance, reflecting the switch from fermentative to aerobic metabolism (Table 1; supplemental Fig. 1). However, the most severely repressed components of the CAC, the 2-oxoglutarate dehydrogenase complex and succinyl-CoA synthetase (sucABCD), were slower to respond, first appearing after 10 min of air exposure (Table 1; supplemental Fig. 1). This could suggest a need for metabolic (2-oxo-glutarate) as well as environmental (O2) signals for transcription of these genes (20) or a requirement for relief of integration host factor-mediated repression of the sucA promoter (21). Furthermore, despite being repressed by FNR and ArcA (22), fumA did not meet the criteria set for responsive transcripts at any of the time points. The abundance of the fumA did increase after culture aeration (1.6-fold after 5 min, 1.9-fold after 10 min, 2.0-fold after 15 min, and 1.1-fold after 60 min); however, these changes were not regarded as statistically significant with p values ranging from 0.32 to 0.82 (supplemental Table 2). It is possible that the anaerobic fumarase, FumB, fulfills the role of FumA during the initial stages of aerobic growth, because FumB activity has been detected when cultures are exposed to 8% oxygen (23). Alternatively, there might be sufficient FumA synthesized under anaerobic conditions to support the aerobic CAC, because significant FumA activity has been detected in anaerobic cultures (23).

The CAC supplies reducing equivalents for the aerobic electron transport chain. E. coli possesses alternative primary NADH dehydrogenases, the non-proton pumping NdhiI (ndhi), and the energy-conserving Ndhal (nuoA-N operon), as well as alternative oxidases, cytochrome bd-type oxidase (cydAB), which has a very high affinity for O2, or cytochrome bo-type oxidase (cyoA-E operon), which has a lower affinity for O2 (reviewed by Gennis and Stewart (2)). The transcription profile data show that ndhi and cyoA-E were enhanced upon culture aeration, suggesting that an electron transport chain beginning with NdhiI and terminating in Cyo is assembled (Table 1; supplemental Fig. 1). In contrast, both nuo and cyd transcripts were less abundant after perturbation of the anaerobic steady state (supplemental Table 2), in accordance with patterns of regulation reported previously (24–26).

Thus, within 10 min of exposure to air, the transcript profile of E. coli was profoundly altered; CAC transcripts were enhanced and, assuming that transcript levels reflect changes in enzyme activity, the left arm of the CAC is switched from the reductive to oxidative direction. The changes in transcript abundance also suggest that the reducing equivalents generated by the CAC are fed into an aerobic electron transport chain via NdhiI to ultimately reduce O2 to water via Cyo (supplemental Fig. 1). These patterns of transcript abundance were retained 15 and 60 min after the introduction of air, except that the oxidative stress-induced aconitase A transcript was detected, presumably to help maintain CAC integrity under conditions where the more labile aconitase B might be vulnerable (27, 28).

The relief of ArcA- and FNR-mediated repression accounts for the increased abundance of CAC and aerobic respiratory chain transcripts upon introduction of air (Table 1) (reviewed by Lynch and Lin (29) and Guest et al. (1)), and the temporal changes observed here are consistent with the kinetics of FNR inactivation by O2 (30). Although FNR is a direct O2 sensor, ArcA primarily responds to the redox status of the quinone pool and the accumulation of fermentation products, such as NADH and \(\Delta\)-lactate (31, 32). Measurement of the relative amounts of NADH and NAD\(^+\) in the absence (NADH:NAD\(^+\) = 0.64) and presence (NADH:NAD\(^+\) = 0.10) of air were similar to those reported previously (33). The decrease in intracellular NADH after the introduction of air suggests that the changes in the abundance of transcripts encoding components of the aerobic electron transport chain are physiologically significant. This would be consistent with a more oxidized quinone pool and the inactivation of ArcA (32).

From the transcript profiling data it appeared that, upon introduction of air, the pdhR-aceE-aceF-lpd operon exhibits a rapid transcriptional response; the sucABCD operon exhibits an intermediate response, and the acnA gene exhibits a slow response; thus these three operons represent the range of regulatory responses observed (Table 1). Single copy lacZ fusions in strain MC4100 (lac) were used to monitor expression of these operons in vivo when anaerobic batch cultures were transferred to aerobic conditions. The reporter strains showed a similar pattern of expression upon aeration to that observed...
Similarly, the transient enhancement (2-fold) of the fermentation product to be utilized as a respiratory substrate. PdhR-mediated activation. This suggests that the introduction of oxygen allows the lactate that was excreted as an anaerobic fermentation product to be utilized as a respiratory substrate.

### TABLE 1

Transcripts encoding glycolysis, citric acid cycle, and respiratory chain proteins that are present in increased abundance after the introduction of air to anaerobic cultures of *E. coli* MG1655

Fold regulation (by at least 2-fold at one or more time points) is the ratio of transcript levels at the indicated time after the introduction of air to the transcript levels at *t* = 0 (the initial anaerobic state; *p* ≤ 0.05). Where no value is shown, there was no significant change in transcript abundance. Relevant regulatory proteins were taken from the EcoCyc website, where assignments of regulation include transcript profiling data; where there is evidence for direct (interaction of a regulator with promoter DNA) regulation of a particular promoter, this is indicated by *. (−) indicates negative regulation; (+) indicates positive regulation; and (−/+ ) indicates negative and positive regulation by the indicated transcription factor. In cases where multiple promoters with different regulation have been recognized, they are distinguished as P1, P2 etc.

| Glycolysis, citric acid cycle, and respiratory chain genes | -Fold up-regulation relative to the | Relevant regulatory proteins |
|-----------------------------------------------------------|---------------------------------|-----------------------------|
| **PdhR** | **Transcriptional state at the indicated times following the introduction of air** | **Proteins** |
| **5 min** | **10 min** | **15 min** | **60 min** | **PdhR**, **FRN** | **CRP** |

| **aceE** pyruvate dehydrogenase (decarboxylase component) | 6.0 | 6.7 | 6.9 | 4.7 | **PdhR**, **FRN** | **CRP** |
| **aceF** pyruvate dehydrogenase(dehydroacetoaceyltransferase component) | 3.6 | 5.7 | 6.1 | 3.2 | **PdhR**, **FRN** | **CRP** |
| **acnA** aconitase A | 3.2 | 3.2 | 2.0 | 2.6 | **ArcA** | **CRP** |
| **acnB** aconitase B | 7.4 | 5.8 | 7.3 | 6.8 | **ArcA** | **CRP** |
| **cycA** cytochrome bo terminal oxidase subunit II | 10.4 | 8.6 | 9.1 | 8.6 | **ArcA** | **CRP** |
| **cycB** cytochrome bo terminal oxidase subunit I | 12.9 | 10.0 | 11.7 | 9.9 | **ArcA** | **CRP** |
| **cycD** cytochrome bo terminal oxidase subunit IV | 9.2 | 7.9 | 7.8 | 7.3 | **ArcA** | **CRP** |
| **cydA** heme o synthase | 5.7 | 6.3 | 6.5 | 5.2 | **ArcA** | **CRP** |
| **gltA** fatty acid transport-pyruvate formate-lyase | 4.4 | 4.0 | 4.7 | 2.7 | **ArcA** | **CRP** |
| **gltB** fatty acid transport-pyruvate formate-lyase | 4.8 | 5.3 | 6.1 | 5.0 | **ArcA** | **CRP** |
| **ipDA** lipoamide dehydrogenase (E3 monomer) | 3.0 | 3.9 | 4.9 | 3.3 | **PdhR**, **FRN** | **CRP** |
| **mdh** malate dehydrogenase | 2.4 | 3.4 | 3.5 | 2.4 | **PdhR**, **FRN** | **CRP** |
| **mgo** malate dehydrogenase (quinone) | 4.8 | 6.9 | 8.6 | 6.6 | **ArcA** | **CRP** |
| **ndh** NADH dehydrogenase II | 2.9 | 4.2 | 5.0 | 3.1 | **FNR** | **CRP** |
| **pdx** pyruvate responsive transcription regulator | 3.5 | 4.6 | 5.0 | 3.0 | **PdhR**, **FRN** | **CRP** |
| **poxB** pyruvate oxidase | 2.3 | 2.2 | 2.4 | 3.7 | **SoxRS** | **MarR** |
| **sdIA** succinate dehydrogenase flavoprotein | 5.5 | 3.7 | 3.5 | 2.1 | **P1**, **ArcA** | **CRP** |
| **sdIB** succinate dehydrogenase iron-sulfur protein | 9.1 | 6.8 | 5.8 | 2.9 | **P1**, **ArcA** | **CRP** |
| **sdIC** succinate dehydrogenase membrane protein | 16.6 | 9.9 | 8.7 | 5.8 | **P1**, **ArcA** | **CRP** |
| **sdID** succinate dehydrogenase membrane protein | 7.8 | 4.6 | 4.3 | 2.8 | **P2**, **CRP** | **P2**, **CRP** |
| **sucA** 2-oxoglutarate dehydrogenase | 1.9 | 3.2 | 3.2 | 2.0 | **P1**, **ArcA** | **CRP** |
| **sucB** dihydroxyacetone phosphate dehydrogenase | 3.6 | 3.6 | 2.1 | **P2**, **ArcA** | **CRP** |
| **sucC** succinyl-CoA synthetase, β subunit | 3.5 | 3.1 | 2.1 | **P1**, **ArcA** | **CRP** |
| **sucD** succinyl-CoA synthetase, α subunit | 4.7 | 2.7 | **P2**, **ArcA** | **CRP** |

in the transcript profiling experiments confirming the order of response of these genes (supplementary Fig. 2).

As expected, the abundances of transcripts associated with anaerobic metabolism, such as terminal reductases, hydrogenases, formate transport-pyruvate formate-lyase, and molybdate cofactor biosynthesis were decreased on aereration (supplementary Fig. 1 and supplemental Table 3). Some transcripts (eno, pgi, pkA, pykA, and pykF) encoding enzymes of the glycolytic pathway also decreased in abundance in the presence of air, consistent with lower glycolytic flux in aerobic compared with anaerobic cultures (34).

**Utilization of Excreted Fermentation Products—**The anaerobic steady-state cultures contained significant amounts of acetate, ethanol, succinate, formate, and lactate (see above). Within 5 min of culture aereration the lctPRD transcripts, encoding a lactate transporter, a regulator, and a lactate dehydrogenase, were increased by ~3–5-fold (supplemental Table 4), presumably due to the relief of ArcA-mediated repression and PdhR-mediated activation. This suggests that the introduction of air allows the lactate that was excreted as an anaerobic fermentation product to be utilized as a respiratory substrate. Similarly, the transient enhancement (2-fold) of the dctA transcript (encoding a dicarboxylic acid symporter) indicates that previously excreted succinate can also be used to feed the CAC during the initial stages of the transition to aerobic conditions (supplemental Table 3). In addition, the 2-oxoglutamate transporter *kdgT* transcript was enhanced (~2.5–3.5-fold) in all samples following aereration (supplemental Table 3). However, there was no transcriptional response related to exploitation of acetate, as the *acs-yjeH-yjgG* (encoding acetyl-CoA synthetase and acetate permease) was unaltered (35). Furthermore, at 15 and 60 min, the *poxB* transcript (encoding pyruvate oxidase, a flavoprotein that converts pyruvate to acetate and CO₂) was present in increased abundance (~2–5-fold; Table 1), suggesting that in the presence of oxygen the potential to generate acetate remains.

**Introduction of Oxygen into Anaerobic Cultures Causes Transient Peroxide Stress and Increased Demand for Iron-Sulfur Cluster Assembly—**Anaerobic metabolism is accompanied by oxidative stress imposed by endogenous generation of reactive oxygen species (36). In *E. coli* the oxidative stress response is coordinated by the transcription factors SoxRS (responding to superoxide) and OxyR (responding to hydrogen peroxide) (reviewed by Storz and Zheng (37); Green and Paget (38)).
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Within 5 min of air exposure the abundance of the soxS transcript is increased ~8-fold (Table 2), indicating activation of the sensor SoxR, which enhances soxS expression, which in turn controls the SoxRS regulon (39). However, only 6 of 66 genes that are activated in response to paraquat-generated superoxide stress (39) were up-regulated here: ahpCF, cyoD, dadX, gtaA, and sdhB (Table 2). To the authors’ knowledge there is no direct evidence for SoxRS binding to the promoter regions of these genes. Furthermore, these transcripts (except dadX) are regulated either by ArcA, FNR, or OxyR, possibly accounting for the increased abundance of these transcripts in the presence of O$_2$. It has been reported that the late responding transcripts acnA, fur, and poxB expression are influenced by SoxRS (40–42), but they are also subject to complex regulation (Table 2). Thus, despite the increased abundance of the soxS transcript, presumably reflecting an increased production of superoxide under aerobic conditions that is sensed by SoxR (43), the level of SoxS remains below the threshold required to trigger expression of the SoxS regulon.

In contrast to the SoxRS regulon, inspection of the 22 OxyR-dependent genes that are up-regulated in response to exogenous hydrogen peroxide (44) revealed that 5, ahpCF, grxA, katG, and trxC, were up-regulated during the initial switch to aerobic metabolism (Table 2). These were followed at the 15- and 60-min time points by the suf (iron-sulfur cluster repair) and fur transcripts (Table 2 and below). As the culture adapted to the presence of O$_2$, some of the highly induced, rapidly responding transcripts decreased in abundance (Table 2). Notably, the grxA and trxC transcripts were much less abundant in the 15- and 60-min samples than at 5 and 10 min (Table 2). The GrxA protein participates in the regulatory feedback loop that controls OxyR activity (45). The progressive decrease in grxA transcript abundance suggests that OxyR is most active during the early stages of adaptation to aerobic metabolism. The temporal changes in the abundances of transcripts associated with oxidative stress are consistent with a burst of peroxide stress upon aeration, followed by enhanced peroxide production under aerobic compared with anaerobic conditions (46, 47). The response times observed here are consistent with those reported for the in vivo oxidation and reduction of OxyR when cultures are exposed to exogenous hydrogen peroxide (48). Thus, there appears to be a subset of OxyR-regulated transcripts (ahpCF, grxA, katG, and trxC) that play particularly important roles in the management of endogenous oxidative stress during the initial phases of adaptation to aerobic metabolism.

In addition to the anti-oxidant activities discussed above, the ArcA- and FNR-repressed tpx (thioredoxin-dependent thiol
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**TABLE 3**

Transcripts encoding proteins involved in methionine biosynthesis and putrescine utilization that exhibit altered abundance after the introduction of air to anaerobic cultures of *E. coli* MG1655

Fold regulation is the ratio of transcript levels at the indicated time after the introduction of air to the transcript levels at *t* = 0 (the initial anaerobic state; *p* ≤ 0.05). Relevant regulatory proteins were taken from the EcoCyc website. Where no value is shown, there was no significant change in transcript abundance. Where there is evidence for direct regulation of a particular promoter, this is indicated by *.*  

| MetH | Regulator | Fold | 5 min | 10 min | 15 min | 60 min |
|------|-----------|------|-------|--------|---------|--------|
| *metA* | homoserine o-succinyltransferase | 5.8 | 4.0 | 3.8 | 2.2 | 1.9 |
| *metB* | o-succinylhomoserine lyase | 3.3 | 2.1 | 2.0 | 1.8 | 1.7 |
| *metC* | cystathionine-1-homocysteine-lyase | 1.6 | 2.4 | 2.4 | 2.7 | 2.7 |
| *metE* | cobalamin-dependent homocysteine transmethylase | 3.3 | 3.8 | 3.0 | 2.3 | 2.3 |
| *metF* | 5,10-methylenetetrahydrofolate reductase | 3.5 | 2.5 | 2.9 | 2.7 | 2.7 |
| *metH* | cobalamin-dependent homocysteine transmethylase | 2.8 | 2.1 | 3.8 | 3.0 | 3.2 |
| *metL* | transcriptional repressor | 2.8 | 3.3 | 3.2 | 3.3 | 3.3 |
| *metK* | S-adenosylmethionine synthetase | 2.8 | 3.3 | 3.2 | 3.3 | 3.3 |
| *metL* | membrane component of o-methionine ABC transporter | 2.8 | 3.3 | 3.2 | 3.3 | 3.3 |
| *metN* | ATP binding component of ABC transporter | 2.8 | 3.3 | 3.2 | 3.3 | 3.3 |
| *metP* | periplasmic binding protein for o-methionine | 2.8 | 3.3 | 3.2 | 3.3 | 3.3 |
| *metR* | transcriptional activator | 2.8 | 3.3 | 3.2 | 3.3 | 3.3 |
| *msrB* | methionine sulfoxide reductase B | 2.8 | 3.3 | 3.2 | 3.3 | 3.3 |
| *slp* | periplasmic sulfate-binding protein | 2.8 | 3.3 | 3.2 | 3.3 | 3.3 |

Putrescine catabolism

| Putrescine metabolism | Enzyme | Fold | 5 min | 10 min | 15 min | 60 min |
|-----------------------|--------|------|-------|--------|---------|--------|
| *argI* | ornithine transcarbamylase | 2.8 | 4.0 | 3.8 | 2.2 | 1.9 |
| *argT* | component of lysine/arginine/ornithine ABC transporter | 2.8 | 2.1 | 2.0 | 1.8 | 1.7 |
| *gadB* | succinate-semialdehyde dehydrogenase; NADP dependent | 2.8 | 2.1 | 2.0 | 1.8 | 1.7 |
| *gabT* | aminobutyrate aminotransferase | 2.8 | 2.1 | 2.0 | 1.8 | 1.7 |
| *psua* | γ-glutamylputrescine synthetase | 2.8 | 2.1 | 2.0 | 1.8 | 1.7 |
| *psuaA* | γ-glutamylputrescine oxidase | 2.8 | 2.1 | 2.0 | 1.8 | 1.7 |
| *psuc* | γ-glutamylaminobutyraldehyde dehydrogenase | 2.8 | 2.1 | 2.0 | 1.8 | 1.7 |
| *psud* | γ-glutamyl-γ-aminobutyrate hydrolase | 2.8 | 2.1 | 2.0 | 1.8 | 1.7 |
| *psueE* | 4-aminobutyrate aminotransferase | 2.8 | 2.1 | 2.0 | 1.8 | 1.7 |
| *yggE* | putrescine-2-oxoglutaric acid aminotransferase | 2.8 | 2.1 | 2.0 | 1.8 | 1.7 |

**Notes:**
- Fold regulation is the ratio of transcript levels at the indicated time after the introduction of air to the transcript levels at *t* = 0 (the initial anaerobic state; *p* ≤ 0.05).
- Regulatory proteins were taken from the EcoCyc website.
- Where no value is shown, there was no significant change in transcript abundance.
- Where there is evidence for direct regulation of a particular promoter, this is indicated by *.*

 Peroxidase) was induced throughout the 60-min time course (Table 2). Furthermore, sodC, an FNR-repressed CuZn superoxide dismutase (49), and osmC (organic hydroperoxidase) were enhanced at the 15- to 60-min time points, whereas the redox-balancing cytoplasmic transhydrogenase *udhA* appeared only at the 5-min point (Table 2).

The *iscRS*-*iscBA*-fdx-*iscX*-pepB-*sseB* operon (50) encodes proteins involved in the assembly of iron-sulfur clusters (reviewed by Barras et al. (51)). The abundances of the *iscRS*-*iscBA*-fdx transcripts were enhanced within 5 min of culture aeration, and this enhancement was maintained (Table 2; supplemental Fig. 1). The amounts of the *iscX*, *pepB*, and *sseB* transcripts also increased within 15 min of culture aeration (Table 2). These transcriptional responses suggest that the switch to aerobic metabolism creates additional demand for de novo iron-sulfur cluster synthesis to satisfy the needs of new iron-sulfur proteins, and to replace damaged iron-sulfur proteins that are required under both aerobic and anaerobic conditions.

Although the *isc* apparatus is considered to be mainly involved in iron-sulfur cluster assembly under conditions of normal aeration, the *sufABCDSE* operon is regarded as more important for iron-sulfur assembly under conditions of oxidative stress (reviewed by Barras et al. (51)). In contrast to the *isc* transcripts, the *suf* operon transcripts appear late in the transition to aerobic growth (Table 2). In addition, the abundance of the *yhiG* transcript, encoding a protein related to IscA, was also increased. Taken together, these data suggest that there is a lower demand for iron-sulfur cluster biosynthesis under anaerobic conditions and that once aerobic growth is established both Isc- and Suf-mediated iron-sulfur cluster repair and assembly are required, although the temporal responses of these two systems are distinct.

Methionine Biosynthetic Genes Are Transiently Induced during Adaptation to Aerobic Conditions—Cobalamin-independent methionine synthase, MetE, is inactivated upon exposure of *E. coli* to peroxide- or diamide-mediated oxidative stress (52, 53). A component of MetE inactivation involves reversible glutathionylation of Cys-645, which is thought to protect the active site from further oxidative damage (52). Oxidative inhibition of MetE results in methionine auxotrophy (52). It is now clear from the data presented here that methionine biosynthesis is also interrupted during adaptation to aeration (Table 3). During the first 15 min following aeration of the cultures the abundances of transcripts encoding methionine biosynthesis proteins increase, consistent with progressive methionine starvation (Table 3). These changes are accompanied by enhanced abundance of transcripts (metNIQ) encoding d-methionine transport proteins at the 10- and 15-min time points and the gratuitous induction of the cobalamin-dependent methionine synthase, MetH, after 15 min of aeration. However, as the cultures adapted to the presence of O2 (60 min), the amounts of met biosynthetic and transport transcripts were lower than.
TABLE 4

Effect of lesions in the fnr and arcA genes on puuA and puuD expression

Cultures of the indicated strains carrying plasmid-based puuA-lacZ or puuD-lacZ fusions (supplemental Table I) were grown at 37 °C until the cultures reached an optical density of 0.4–0.6 under aerobic (10 ml of medium in 250-ml flask shaken at 250 rpm) or anaerobic (sealed bottles) conditions in L broth supplemented with 0.2 (w/v) glucose. Promoter activities were determined by measuring β-galactosidase activities. Values are means ± S.D. of triplicate assays from three independent cultures.

| Strain          | puuA-lacZ | puuA-lacZ | puuD-lacZ | puuD-lacZ |
|-----------------|-----------|-----------|-----------|-----------|
|                 | Aerobic   | Anaerobic | Aerobic   | Anaerobic |
| MC1000 (parent) | 5975 ± 540| 1210 ± 110| 6755 ± 605| 2815 ± 110|
| JRG1728 (fnr)   | 4630 ± 290| 2215 ± 180| 5710 ± 505| 2910 ± 140|
| MC4100 (parent) | 4895 ± 410| 1251 ± 60 | 5470 ± 270| 2545 ± 180|
| JRG5351 (arcA)  | 14,777 ± 1015| 27,300 ± 2120| 12,600 ± 2055| 21,990 ± 2075|

FIGURE 1. Changes in DNA supercoiling during the transition between anaerobic and aerobic growth. Supercoiling of plasmid pGEM3Zf(+) isolated at the indicated times after aeration of anaerobic cultures of MG1655. Gels and buffers contained 25 μg ml⁻¹ chloroquine. Each lane was loaded with 0.6 μg of plasmid DNA. More relaxed topoisomers migrate furthest in the gels.

FIGURE 2. Real time PCR expression of iscR, metF, puuD, and trxC during the transition to aerobic conditions. Real time quantitative reverse transcription-PCR analysis was used to investigate changes in abundance of the indicated transcripts (iscR, metF, puuD, and trxC) during exposure of anaerobic cultures of E. coli to air. Mean values of duplicate assays are shown; bars show standard deviation (in most cases within the sizes of the data points). The mRNA levels were normalized to gyrA as an internal control. The amounts of each transcript were then referenced to the amounts present at t = 0 (i.e., the anaerobic starting state), which were arbitrarily set to 1 (dashed line).

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those present in the anaerobic steady state (Table 3). The simplest explanation for these changes in transcript profiles is that the introduction of air into the anaerobic cultures caused inactivation of MetE, leading to methionine starvation and MetR-mediated activation of met genes (54). Based on the OxyR response (see above), within 60 min of aeration the redox balance of the cytoplasm is restored allowing MetE reactivation. At this point the supply of methionine exceeds demand and is sensed by MetR and MetJ. Consequently, the abundances of transcripts encoding methionine biosynthesis proteins fall below the amounts present in the anaerobic starting state. It is notable that methionine biosynthesis is also a casualty when cultures are exposed to nitrosative stress (12), emphasizing the links between oxidative and nitrosative stress responses. In addition, sustained enhanced abundance of the msrB transcript, encoding methionine sulfoxide reductase B, suggests that methionine oxidation poses problems during the transition to aerobic growth (Table 3).

Transcripts Encoding a Putrescine Degradation Pathway Are Enhanced in the Transition to Aerobic Conditions—The intracellular concentration of putrescine is ~20 mM for mid-exponential phase E. coli cells (55). The transition to aerobic growth was accompanied by the sequential appearance of transcripts associated with putrescine degradation (Table 3) (56). This catabolic pathway consists of the ATP-dependent γ-glutamyltranspeptidase of putrescine (PuuA), the O₂-dependent conversion of γ-glutamylputrescine to γ-glutamyl-γ-aminobutyraldehyde (PuuB), subsequent dehydration to yield γ-glutamyl-γ-aminobutyrate (PuuC), followed by hydrolysis to 4-aminobutyrate (PuuD), and conversion to succinate via succinate semialdehyde (PuuE and GabD) (supplemental Fig. 1). The succinate produced can then be used to feed the CAC, thereby supporting aerobic metabolism. The order in which the transcripts were detected after culture aeration could be related to the architecture of the puu gene cluster. The puuA and puuD transcripts appeared after 5 min and are transcribed from divergent promoters as follows: puuA as a monocistronic mRNA, and puuD as a puuD-puuR bicistronic mRNA that includes the predicted transcription regulator PuuR. There are credible FNR (ttgataacgagcgg) and ArcA (gttctatta) sites in the puuA–D intergenic region, suggesting the possibility of de-repression in the presence of O₂. In vivo transcription assays using plasmid-based puuA-lacZ and puuD-lacZ fusions revealed that both puuA and puuD promoters were less active under anaerobic, compared with aerobic, conditions (Table 4). In an fnr mutant anaerobic expression of puuD was unaffected, whereas expression of puuA was ~2-fold greater than that determined for the parent strain (Table 4). In an arcA mutant both puuA and puuD promoters exhibited enhanced activity under aerobic and anaerobic conditions (Table 4). This is consistent with repression of puuA and puuD...
expression in the absence of oxygen mediated by ArcBA, with an additional contribution by FNR at the puuA promoter.

After 10–15 min the puuCBE transcripts appear, and these are expressed as a single mRNA from a promoter located downstream of puuR. This might suggest that the activities encoded by the puuA and puuD transcripts promote expression of the puuCBE operon, perhaps via the predicted transcription factor PuuR. In addition, the expression of ygiG, a putrescine:2-oxoglutaric acid aminotransferase, exhibited unusual behavior, appearing at 10 and 60 min but not at 5 or 15 min (Table 3).

Overall, these dynamics suggest the presence of complex circuits that regulate putrescine degradation in response to O₂ and other metabolic signals.

The absence of putrescine in the culture medium indicates that any putrescine degraded during the anaerobic-aerobic transition is likely to be intracellular, a view supported by the absence of induction of the puuD transcript encoding the putrescine importer (PuuP). Intracellular concentrations of putrescine have been reported to change in response to culture aerations (55). The implied degradation of putrescine, which is known to offer some protection against the effect of reactive oxygen species (57–59), is perhaps surprising in the anaerobic-aerobic transition, and its degradation might cause some aspects of the oxidative stress response discussed above by depleting one sink for reactive oxygen species. However, putrescine also alters DNA topology (60). Thus, to investigate whether the increased abundance of the puu gene cluster transcripts correlated with the extent of DNA supercoiling, samples of MG1655 and the corresponding puuA mutant carrying the plasmid pGEM3Zf(+) were obtained before and during exposure to air. Gel electrophoresis to separate plasmid topoisomers revealed that for MG1655 plasmid supercoiling initially increased, but the DNA became more relaxed at the 15- and 60-min time points (Fig. 1). However, although a strong phenotype was not evident in the puuA mutant, the changes in DNA topology were consistently delayed (Fig. 1). Thus, the inability to degrade putrescine, via the Puu pathway, correlates with the dynamics of remodeling DNA topology during the transition to aerobic growth. This is a potentially important observation because DNA topology changes are involved in the transcription of a range of genes involved in a variety of cellular processes (reviewed by Travers and Muskhelishvili (61)).

Putrescine is also important as an osmoprotectant, and putrescine content is inversely proportional to the osmotic strength of the culture medium (62). Thus, the depletion of the intracellular putrescine pool may be reflected in the observed increased abundance of the otSA, otSB, osmY, and prop transcripts encoding functions related to osmotic protection (supplemental Table 2).

**Real Time PCR—Changes in the abundance of the iscR, metF, puuD, and trxC transcripts as representatives of the responses discussed above were investigated by real time PCR. The results (Fig. 2) confirmed the changes in transcript abundance observed in the transcript profiling experiments.**

**Transient Induction of Genes of Unknown Function**—The switch to aerobic growth was accompanied by enhanced abundance of 27 transcripts from genes that possess domains of unknown function that are conserved in other bacteria. Among these there are 10 transcripts that were expressed transiently during the initial stages of adaptation to aerobic growth. The abundances of five of these (yacL, ybdN, ygbf, yhjG, and yzgL) were maximal at the 5-min time point, suggesting that they are involved in the initial phase of adaptation (Table 5). Interestingly, in the context of this study, the yacL gene is located immediately downstream of acnB, encoding the CAC enzyme aconitate B (AcnB). Aconitate B possesses a relatively unstable iron-sulfur cluster, and the apo-form of the protein acts as a post-transcriptional regulator (63–65), and the yacL transcript was identified as an apoAcnB target.³ Bacterial two-hybrid analysis revealed apparently stronger interaction between holoAcnB and YaCL than between apoAcnB and YaCL in vivo (supplemental Fig. 3). The interaction point in AcnB was mapped to the N-terminal region, which contains the HEAT-like protein interaction domain, domain 5 (66). Interaction in vivo was not observed between YaCL and AcnB domains 1–2–3 (supplemental Fig. 3). Thus, in addition to its putative role in transcript elongation (67), it is possible that YaCL might mod-

³ Y. Tang and J. Green, unpublished data.

### Table 5

| Gene     | Predicted function/conserved domains | -Fold change relative to the anaerobic steady state at the indicated times following the introduction of air | Position of potential FNR- and/or Arc-binding sites |
|----------|-------------------------------------|------------------------------------------------|-------------------------------------------------|
| yacL     | Conserved domain of unknown function | 2.1                                           | None                                            |
| ygbf     | Conserved domain of unknown function | 2.0                                           | None                                            |
| yhjG     | Homology to AsmA family              | 3.7                                           | None                                            |
| ygbf     | Conserved domain of unknown function | 2.3                                           | None                                            |
| ybdN     | Phosphoenolpyruvate phosphatase domain| 2.2                                           | ArcA, −53.5 (2); FNR, −143.5 (2), −420.5 (1)   |
| ycgW     | Conserved domain of unknown function | 3.7                                           | ArcA, −103.5 (1); −257.5 (2); FNR, −157.5 (2)  |
| ybdD     | Conserved domain of unknown function | 4.6                                           | None                                            |
| yace     | Function unknown, phosphatase activity| 5.0                                           | None                                            |
| yciO     | Possesses a domain shown to bind double-stranded RNA | 2.0 | FNR, −111.5 (2); −321.5 (2) |
| ydeL     | Function unknown, domain similarity to CinA | 2.1 | FNR, −489.5 (1) |
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TABLE 6
Effect of oxyR, metR, and puuA mutations on the ability of E. coli to adapt to aerobic conditions

Anaerobic cultures of parent and mutant strains were combined such that the mixtures contained similar colonies forming units. Aliquots (5 ml) of the mixtures were grown at 37 °C under anaerobic (250 ml conical flask shaken at 250 rpm) and aerobic conditions (sealed bottles under an oxygen-free nitrogen atmosphere). After 2 h, the total numbers of colony forming units were determined by serial dilution and plating on 1-agar and the numbers of mutant colony forming units by plating on 1-agar containing kanamycin (30 mg liter−1). The competitive index (mutant/parentaerobic × mutant/parentanaerobic) should be close to 1.00 if the mutant is unimpaired and <1.00 if the mutant is impaired in the transition to aerobic conditions. The data are the mean (±S.D. of three independent experiments). Where indicated methionine (1 mM) or putrescine (20 mM) was added to the mixed cultures.

| Test strain | Competitive index | Aerobic | Aerobic plus methionine | Aerobic plus putrescine |
|-------------|-------------------|---------|------------------------|------------------------|
| oxyR        | 0.44 ± 0.07       | 1.00 ± 0.06 | 0.81 ± 0.11 |
| metR        | 0.46 ± 0.15       | 1.00 ± 0.06 | 0.81 ± 0.11 |
| puuA        | 0.27 ± 0.04       | 1.00 ± 0.06 | 0.81 ± 0.11 |

ulate the enzymatic and regulatory functions of AcnB during adaptation to aerobic conditions.

Two transcripts, ycgW and ybdJ, exhibited maximal abundance at the 10-min point (Table 5). There are credible ArcA and FNR-binding sites upstream of the ycgW coding region that might account for the observed regulation. After 15 min the amounts of the yael, yciO, and ydeI transcripts were maximal, and there are putative FNR sites located upstream of the latter two transcripts. The transient induction of these genes of unknown function suggests that, although their steady-state expression is similar under aerobic and anaerobic conditions, they encode functions that provide E. coli with a competitive advantage allowing rapid adaptation when faced with anaerobic-aerobic transitions. Further experiments will be needed to test this hypothesis.

The Ability of metR, oxyR, and puuA Mutants to Transist between Anaerobic and Aerobic Conditions Is Impaired—Transcript profiling suggested that peroxide stress, methionine supply, and putrescine degradation are important during the transition to aerobic conditions. To test whether the changes in transcript abundance reported above are physiologically significant, the ability of oxyR, metR, and puuA mutants to adapt to aerobic conditions was tested in competitive index studies in which anaerobic cultures containing similar numbers of parent and mutant bacteria were either transferred to aerobic conditions or maintained under anaerobic conditions. The data obtained indicated that the mutants were impaired in the ability to adapt (Table 6). For the metR mutant this defect could be complemented by the addition of methionine (1 mM). For the parent versus puuA experiments, the addition of putrescine (20 mM) during the transition to aerobic conditions improved the relative competitiveness of the mutant by impairing adaptation of the parent (Table 6). These data are consistent with the proposal that methionine biosynthesis and putrescine degradation are involved in the processes leading to efficient adaptation to aerobic conditions.

Conclusions—Investigating transcriptome dynamics as anaerobic cultures of E. coli transit to aerobic growth has revealed that within 10 min the transcriptional switch from anaerobic to aerobic central metabolism is largely complete, mediated by the well established action of FNR and ArcBA in response to oxy-
gen availability. However, for the first time significant roles are revealed for peroxide stress management, methionine biosynthesis and an oxygen-dependent pathway for putrescine degradation, during the transition to aerobic conditions. The observed OxyR-mediated oxidative stress response is consistent with previous reports that show that fumarate reductase produces superoxide in the presence of O₂, and that sufficient superoxide dismutase is present in anaerobic cells to produce hydrogen peroxide and thus activate OxyR (43). The changes in abundance of transcripts associated with methionine biosynthesis are consistent with inactivation of the cobalamin-independent methionine synthase MetE (52, 53) and the combined responses of the regulators MetR and MetJ. The observed enhanced abundance of transcripts encoding enzymes for putrescine degradation was less predictable but is accounted for at least in part by inhibition of ArcA-mediated repression of puuA and puuD expression. Control of putrescine degradation by oxygen availability is potentially very significant, because of the effects on DNA topology that influence transcription of genes involved in a variety of cellular processes (61). Thus, it is suggested that the changes in transcript profile reported here maintain competitiveness during the naturally occurring anaerobic-aerobic transitions experienced by enteric bacteria, and although many questions have been raised, the experiments provide new insights into the dynamics of the complex metabolic remodeling that occurs during the transition to aerobic growth and have generated new hypotheses to stimulate future investigations.

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