Transition of *Escherichia coli* from Aerobic to Micro-aerobic Conditions Involves Fast and Slow Reacting Regulatory Components

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**Abstract**

Understanding life at a systems level is a major aim of biology. The bacterium *Escherichia coli* offers one of the best opportunities to achieve this goal. It is a metabolically versatile bacterium able to respond to changes in oxygen availability. This ability is a crucial component of its lifestyle, allowing it to thrive in aerobic external environments and under the oxygen-starved conditions of a host gut. The controlled growth conditions of chemostat culture were combined with transcript profiling to investigate transcription dynamics during the transition from aerobic to micro-aerobic conditions. In addition to predictable changes in transcripts encoding proteins of central metabolism, the abundances of transcripts involved in homeostasis of redox-reactive metals (Cu and Fe), and cell envelope stress were significantly altered. To gain further insight into the responses of the regulatory networks, the activities of key transcription factors during the transition to micro-aerobic conditions were inferred using a probabilistic modeling approach, which revealed that the response of the direct oxygen sensor FNR was rapid and overshot, whereas the indirect oxygen sensor ArcA reacted more slowly. Similarly, the cell envelope stress sensors RpoE and CpxR reacted rapidly and more slowly, respectively. Thus, it is suggested that combining rapid and slow reacting components in regulatory networks might be a feature of systems in which a signal is perceived by two or more functionally related transcription factors controlling overlapping regulons.

*Escherichia coli* is a metabolically versatile bacterium that is able to grow in the presence and absence of oxygen. To achieve this, it exploits a flexible biochemistry in which aerobic respiration is preferred to anaerobic respiration, which in turn is preferred to fermentation. The preference for aerobic respiration reflects the relative energetic efficiencies of the alternative metabolic modes (1). To exploit the energetic benefits conferred by aerobic respiration, *E. coli* has two alternative quinol oxidases, cytochrome *bo*$_3$ and cytochrome *bd* (2). Cytochrome *bo*$_3$ is a heme-copper protein that is synthesized under aerobic conditions and has a lower affinity for O$_2$ than the heme protein cytochrome *bd*, which has a very high affinity for O$_2$ and is synthesized under micro-aerobic conditions (3–5). In a further adaptation to lower O$_2$ concentrations, the role of the pyruvate dehydrogenase complex (PDHC), which oxidizes pyruvate to acetyl-CoA and CO$_2$, is progressively taken over by pyruvate formate-lyase (PFL), which converts pyruvate to acetyl-CoA and formate (1). These adaptations allow the bacteria to exploit the relatively low levels of O$_2$ present under micro-aerobic conditions and maintain redox balance.

Adaptation to changes in O$_2$ availability is regulated at the level of transcription by two well characterized systems, FNR and ArcBA (6–9). FNR is a direct O$_2$ sensor (10), whereas the ArcBA two-component system senses O$_2$ availability indirectly by monitoring the redox state of the quinone pool (11). FNR and ArcBA cooperate to regulate some operons, such as *cydAB* (encoding cytochrome *bd*) and *focA-pflB* (encoding PFL) (5, 12). Under aerobic conditions expression of *cydAB* is minimal, but as O$_2$ availability decreases, ArcA binds at the *cydAB* promoter to facilitate maximal expression under micro-aerobic conditions (5). As O$_2$ availability decreases further, FNR binds the *cydAB* promoter to repress expression (5). Similarly, under aerobic conditions expression of *pflB* is minimal, but as O$_2$ availability decreases, ArcA and FNR bind to the *focA-pflB* promoter region to activate expression (12). Thus, the transcriptional outputs are dependent on the relative amounts of active transcription factors (in these cases FNR and ArcA) and their regulatory strengths. It is difficult to determine regulatory activities of transcription factors experimentally, but they can be inferred using models where information on network connectivity and time-resolved transcript profiling is available. Such models promise to provide new insight into the responses of regulatory networks in changing environments (13).

In the present work, changes in the *E. coli* transcriptome were monitored as aerobic chemostat cultures adapted to micro-aerobic conditions. It is shown that, as well as predictable changes in transcripts encoding proteins involved in central metabolic processes, the bacteria respond to changes in metal ion physiology and to cell envelope stress during the transition to micro-aerobic growth. Modeling transcriptome...
dynamics revealed that the responses to lower O₂ availability and cell envelope stress involved fast and slow reacting components.

EXPERIMENTAL PROCEDURES

Bacterial Strains and Growth Conditions—Strains of E. coli and plasmids used in this study are listed in supplemental Table S2. It should be noted that E. coli strains do not necessarily respond identically to environmental changes because of differences in their genetic make up. Therefore, in all cases, comparisons were made using the appropriate parent and mutant combinations (supplemental Table S2). 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 liters capacity) with culture agitation speed constant at 300 rpm, and the temperature maintained at 37 °C. Oxygen levels were monitored, and the pH was maintained at 7.2 ± 0.2. Evans-defined growth medium (14) was used with glucose (15 mm) as the carbon source. Steady state cultures were established at a dilution rate of 0.2 h⁻¹. Fully aerobic conditions were maintained by sparging the cultures with air (0.4 liters min⁻¹). The switch to micro-aerobic conditions was achieved by switching off the air sparging the culture.

For competitive index experiments approximately equal numbers of parent (W3110) and yfiD (JR4403; 15) strains were mixed and used to inoculate (1:100) 10 ml of Evans-defined medium (14) with glucose (30 mmx) as the carbon source. The cultures were incubated at 37 °C under 20, 7, or 0% O₂ atmospheres for 16 h. The number of colony forming units (cfu) on L agar and L agar containing kanamycin (30 mg l⁻¹) were then measured. Competitive index (CI) values were calculated as follows: mutant anaerobic/parent anaerobic: mutant aerobic/parent aerobic.

The effects of mutations in key regulators on gene expression were determined using promoter-lacZ fusions (16) of cultures grown under the indicated conditions in L broth (17) according to Miller (18). Oxygen transfer to cultures was measured as described (19). The phenotype of the ompW mutant was investigated using Biolog phenotype microar- rays (Biolog, Hayward CA).

Transcript Profiling—Transcript profiling and data analysis were as described by Partridge et al. (20). Briefly culture samples (10 ml) were flushed from the chemostat directly into RNA Protect (Qiagen) before total RNA purification using a RNeasy Midi kit (Qiagen). Equal quantities of RNA from control (aerobic steady state) and experimental (5, 10, 15, and 60 min lowered exposure to air) cultures were labeled using Cy3-dCTP or Cy5-dCTP. For each E. coli K12 OciChip (Ocimum Biosolutions, product number 2140-000000), dye swap experiments were done for each pairing with two biological replicates (separate chemostat runs).

Data were analyzed using Imagene, version 5.1 and GeneSight version 4 (Biodiscovry Inc) as previously described (20). Genes showing greater than 2-fold changes in transcript abundance at one or more time points with a p value of ≤ 0.05 (as determined by a Student’s t test) were deemed to be differentially regulated.

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Real Time PCR—Reactions and control samples (using the gene-specific primers listed in supplemental Table S2) were assembled and analyzed as previously described (20).

Transcript Mapping by 5’-RACE PCR—RNA was isolated from anaerobic cultures using Qiagen RNeasy mini kits according to the manufacturer’s instructions. The ompW and fnb transcript starts were identified using 2 μg of E. coli RNA per RACE reaction according to the manufacturer’s instructions (Roche Applied Science) with the oligonucleotides listed in supplemental Table S2.

Modeling Transcription Factor Activities—The response of the regulatory network during the transition from aerobic to micro-aerobic conditions was analyzed using a probabilistic model (13). The model adopts a log-linear approximation to the kinetics of the regulatory system where the transcription profiles are modeled as sparse linear combinations of the transcription factor activity profiles. Assumptions are incorporated as prior probability distributions on the active transcription factor profiles and the gene-specific intensity with which they regulate their targets. These are, essentially, a continuity assumption on the activity profiles (encapsulated in the Markov chain structure of the prior distribution) and equal prior probability of repression and activation. The sparsity of the model is provided by the structure of the regulatory network. The gene expression data is used to infer transcription factor activity profiles and the gene specific regulatory intensities using Bayes’ theorem. A more detailed description is provided under supplemental materials.

RESULTS AND DISCUSSION

Global Transcriptional Responses during the Transition from Aerobic to Micro-aerobic Conditions—Previously we have combined chemostats with transcript profiling to investigate the dynamics of gene regulation in E. coli during the transition from anaerobic to aerobic growth (20). The use of chemostat cultures allows very reproducible initial states to be established in which important parameters such as growth rate, pH, O₂ tension, and growth medium composition can be controlled and measured. Thus, the use of chemostat cultures has been shown to offer major benefits for transcriptomic, proteomic, and metabolomic studies (20, 21, 22), resulting in greater reproducibility of analyses between different laboratories, in comparison to the same studies using batch cultures (23). Nevertheless, a criticism of the use of continuous culture in studies of this type has been the potential to select for loss-of-function rpos mutations, particularly at low growth rates (7). However, published evidence suggests that this does not occur under anaerobic conditions (24) or in E. coli MG1655 (25). In the present work we combine chemostat cultures of E. coli MG1655 with transcript profiling to investigate transcriptional changes during the transition of glucose-limited chemostat cultures from aerobic (100% air saturation, ~210 μl dissolved O₂) to micro-aerobic conditions (O₂ transfer rate to the culture of 12 ± 0.5 mmol min⁻¹ liter⁻¹). The choice of glucose as the carbon source will ultimately allow the full range of metabolic modes (aerobic respiration, anaerobic respiration and fermentation) to be investigated without the need to change the carbon source, but will affect the responses of transcripts such as ansB,
that are co-dependent on the cyclic-AMP receptor protein for expression. The dilution rate chosen (0.2 h\(^{-1}\)) was lower than the \(\mu_{\text{max}}\) under micro-aerobic conditions, thus allowing the cultures the potential to respond to changes in O\(_2\) availability without changes in growth rate. Nevertheless, any temporary changes in growth rate during adaptation to the new conditions forms an integral component of the response and will be reflected in the transcriptome. Samples from the aerobic steady-state culture were rapidly flushed into RNA protect (Qiagen) for isolation of total RNA. Switching off the air that was sparging the culture perturbed the aerobic steady-state. Samples were obtained for RNA preparation 5, 10, 15, and 60 min after the air supply to the culture was lowered. The corresponding labeled cDNAs were synthesized and used to probe E. coli K12 arrays (Ocimum Biosolutions). For initial analysis of the data a significant change in transcript abundance was defined as \(\geq 2\)-fold (\(p \leq 0.05\)) at one or more of the time points analyzed. The full dataset is available (GEO accession number GSE6644).

Lowered O\(_2\) Availability Leads to Predictable Changes in Transcripts Encoding Proteins of Central Metabolism—Lowering the supply of O\(_2\) to E. coli cultures should have predictable effects on the transcription of genes involved in central metabolism. In accord with previous work, the transcript profiling experiments are consistent with the function of the PDHC being partially replaced by PFL; repression of citric acid cycle genes and replacement of succinate dehydrogenase by fumarate reductase; induction of a route from oxaloacetate to fumarate via aspartate; expression of the cydAB encoded terminal oxidase; and induction of anaerobic reductases (26) (Fig. 1A and Table 1). Notably, the terminal reductases NarGHIJ and NirBCD are transiently induced, exhibiting maximal transcript abundances after 10–15 min of O\(_2\) depletion, even in the absence of nitrate or nitrite (Table 1).

The yfiD transcript, encoding a protein that repairs oxygenolytically cleaved PFL under micro-aerobic conditions (27), was markedly increased in abundance (Table 1). Maximal yfiD expression under micro-aerobic conditions is achieved by the
TABLE 1
Transcripts encoding proteins of central metabolism that are present in altered abundance after lowering the supply of air to cultures of E. coli MG1655

The table was compiled from references in the text and the EcoCyc database.

| Operon* | Product | Fold change in abundance relative to the aerobic steady-state at the indicated times (min) following the switch to micro-aerobic conditions† | Relevant regulatory proteins‡ |
|---------|---------|---------------------------------------------------------------------------------------------------------------------------------|------------------------------|
| pdhR-acmE-acmF-lpdA | PDHC | 5.4 4.7 | FNR (—/+) PdhR (—) |
| acnB | Aconitase B | 2.3 | ArcA (—) |
| aspA-dcuA | Aspartase | 2.2 | FNR (+) |
| aspC | Aspartate transaminase | 1.4 | |
| cydA-cydB | Cytochrome bd-1 oxidase | 2.9 | ArcA (+) FNR (—) |
| fociA-fjl | Formate transport/PFL | 2.3 | ArcA (+/-) FNR (‡) |
| frda-frdB-frdC-frdD | Fumarate reductase | 3.0 | FNR (+) |
| gltA | Citrate synthase | —2.5 | ArcA (—) |
| hypA-hypB-hypC-hypD-hypE | Hydrogenase 3 | 4.2 | FNR (+) |
| narG-narH-narI-narJ | Nitrate reductase | 4.1 | FNR (+) |
| nirB-nirC-nirD-cysG | Nitrile reductase | 5.4 | FNR (+) |
| sdhC-sdhD-sdhA-sdhB-sucA-sucB-sucC-sucD | Succinate dehydrogenase/succinyl-CoA synthetase | —11.5 | ArcA (–/+ FNR (—) |
| yfiD | PFL repair protein | 5.9 | ArcA (+) FNR (+/-) CpxR (—) |

*The data shown are for the first gene in the operon unless otherwise indicated by bold font.
† Fold change (by at least 2-fold at one or more time-points) is the ratio of transcript levels at the indicated time to the transcript levels at t = 0 (the initial aerobic state; p ≤ 0.05).
‡ Relevant regulatory proteins are indicated; (—) denotes negative regulation, (+) positive regulation, and (+/-) negative and positive regulation.

O₂-responsive transcription factor FNR binding to tandem sites in yfiD promoter, mediating activation from the downstream site and repression from the upstream site (28). ArcA antagonizes FNR repression (15). The increased abundance of the yfiD transcript, which was confirmed by RT-PCR (supplemental Fig. S1), is consistent with the micro-aerobic status of the cultures after lowering the air supply.

The phenotype of the yfiD mutant was analyzed in competitive index experiments, to determine whether the changes in transcript abundance were reflected in culture physiology. An index of <1.0 indicates that the mutant is impaired compared with the parent. For micro-aerobic conditions, the value was 0.12 ± 0.03; for anaerobic conditions the value was 0.65 ± 0.11 (mean ± S.D., n = 6). Thus, growth of the yfiD mutant was significantly impaired under micro-aerobic conditions compared with the parent, suggesting that the changes in transcription are physiologically relevant.

FNR and ArcA are the major regulators responsible for adaptation of central metabolism to changes in O₂ availability (Table 1). To gain further insight into this regulatory network, a probabilistic model was used to infer FNR and ArcA activity profiles during the transition from aerobic to micro-aerobic conditions. The activity profiles show that FNR (Fig. 1B, blue line) responds rapidly to the change in culture aeration, whereas ArcA (Fig. 1B, red line) responds more slowly. Furthermore, it is inferred from the model that FNR activity peaks after 10 min and then significantly decreases as time proceeds, while the activity of ArcA increases up to 15 min and then stabilizes. The delay in the ArcBA response compared with FNR might reflect the signals perceived by these two transcription factors; FNR is a direct O₂ sensor and responds rapidly, whereas ArcBA monitors O₂ indirectly, via the redox state of the quinone pool, and responds more slowly. Moreover, arcA expression is regulated by FNR (29).

Interruption of the O₂ Supply to Aerobic Cultures Changes Metal Ion Physiology—Metal ion homeostasis is important for maintaining viability because many metal ions are not only essential, but also toxic. Switching E. coli cultures from aerobic to micro-aerobic growth conditions increased the abundance of transcripts involved in copper management (Table 2). Under aerobic conditions the CopA/CueO system is the major contributor to copper tolerance (30). CopA removes copper from the cytoplasm to the periplasm where toxic Cu(I) is oxidized to the less toxic Cu(II) by the multi-copper oxidase, CueO, in a copper- and O₂-dependent reaction. Expression of copA and cueO is regulated by CueR, but only copA responded to lower O₂ availability, exhibiting maximal abundance 10 min into the switch to micro-aerobic conditions (Table 2). Thus, copA transcript abundance during the transition was similar to the inferred activity of FNR (see above). Analysis of the copA promoter revealed a potential FNR site (cTGATGCAAATCGA), with only two mismatches (shown in lowercase) compared with the consensus. This site is centered at —93.5 relative to the copA transcript start (31), suggesting that copA has a class I FNR-dependent promoter, which was confirmed by in vivo transcription using a single copy copA-lacZ fusion that responded to O₂ availability in an FNR-dependent manner (supplemental Table S1). Thus, the model correctly predicted that FNR regulates copA.

The moaABCDE operon encodes proteins required for molybdenopterin biosynthesis and, like copA, is CueR-regulated (31). Analysis of moa transcripts revealed the presence of a copper-repressed promoter upstream of moaA and a copper-inducible transcript originating upstream of moaB (31). During the transition to micro-aerobic conditions the moaA transcript was present in increased abundance (Table 2). However, the regulation observed here is not consistent with CueR-mediated repression of moaA and copper-mediated activation of moaB (31). Thus like copA, the increased abundance of moa transcripts is most likely explained by FNR-mediated activation of the moa promoter in response to lower O₂ availability (32).

In contrast to CopA, the CusCFBA transporter moves copper ions directly to the external milieu (30). Expression of the cusCFBA operon is controlled by the Cu(I)-responsive two-
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### Table 2

Transcripts encoding proteins with known or predicted roles in metal ion homeostasis that are present in altered abundance after lowering of the air supply to cultures of *E. coli* MG1655

| Operon* | Product | Fold change in abundance relative to aerobic steady-state at indicated times (min) following switch to micro-aerobic conditions* | Relevant regulatory proteins* |
|---------|---------|---------------------------------------------------------------------------------|------------------------------|
| feoAB   | Copper translocating P-type ATPase | 2.2 5.2 3.1 3.9 | CueR (+) FNR (+) |
| cuSCuf-cuSB-cuSA | Cus copper efflux system | 2.1 2.5 2.8 3.5 | CueR (+) |
| cutR-cusS | Two-component regulator of Cus | 1.7 1.9 2.4 2.4 | CueR (+) |
| cutC | Copper homeostasis protein | 2.2 2.0 1.5 | RpoE (+) |
| feoA-feoB-yhgG | Ferrous ion transporter | 4.0 7.5 6.2 4.7 | Fur (-) FNR (+) |
| ftnB | Predicted ferritin | 3.7 2.6 3.7 | RpoE (+) |
| yhgG | Molybdopterin biosynthesis protein | 2.9 3.1 2.2 | FNR (+) CueR (-) |

*The data shown are for the first gene in the operon.

* Fold increase (by at least 2-fold at one or more time-points) is the ratio of transcript levels at the indicated time after interrupting the air supply to the transcript levels at \( t = 0 \) (the initial aerobic state; \( p \leq 0.05 \)).

* Relevant regulatory proteins are indicated; (-) negative regulation, (+) positive regulation by the indicated transcription factor.

The table was compiled from this work, references in the text, and the EcoCyc database.

The changes in transcript profiles indicate that during the transition from aerobic to micro-aerobic conditions, metal ion physiology is perturbed, and that FNR plays an important role in managing the uptake and efflux of the redox-active metals copper and iron.

Lower \( O_2 \) Availability Enhances the Abundance of Transcripts Associated with Cell Envelope Stress—Ten minutes after switching to micro-aerobic conditions, transcripts controlled by the cell envelope stress regulators CpxR or RpoE (35–37)
increased in abundance (Table 3 and Fig. 2A). Modeling the activity profiles of CpxR and RpoE suggested that RpoE responds rapidly to decreased O2 availability, exhibiting maximal activity after 15 min, and lower activity after 60 min (Fig. 2B). In contrast, CpxR did not respond initially (0–5 min), but then its activity increased and stabilized after 15 min (Fig. 2B). The profiles of the transcripts in Table 3 did not match the inferred activities of any of the individual regulators analyzed above. This suggested that these transcripts are controlled by multiple transcription factors with differential effects during the transition. For example, a potential CpxR binding motif (GcAAAAAAATGTAAA) has been identified in the yfiD-yung intergenic region (38) and the activity of the FNR- and ArcA-regulated yfiD promoter was ~1.5-fold lower in a cpxR mutant under micro-aerobic and anaerobic conditions (supplemental Table S1).

### Table 3

| Operons       | Products                      | Fold increase in abundance relative to the aerobic steady-state at the indicated times (min) following the switch to micro-aerobic conditions | Relevant regulatory proteins |
|---------------|-------------------------------|---------------------------------------------------------------------------------------------------------------------------------|-------------------------------|
| cpxP          | Negative regulator of the Cpx response | 2.8 2.0 2.0                                                                                                                   | CpxR (+)                      |
| ecfI          | Possible extracytoplasmic function | 2.2                                                                                                                           | CpxR (+) RpoE (+)             |
| ecfL          | Possible extracytoplasmic function | 2.2 2.0 1.6                                                                                                                   | RpoE (+)                      |
| ftnB          | Ferritin-like protein          | 3.7 2.6 3.7                                                                                                                   | CpxR (+) RpoE (+)             |
| htrA          | Periplasmic serine protease    | 3.5 2.7 1.6                                                                                                                   | CpxR (+)                      |
| lpxP          | Palmitoleoyl acyltransferase   | 2.4 1.8                                                                                                                       | RpoE (+)                      |
| ompW          | β-Barrel protein               | 4.0 7.7 10.7 28.3                                                                                                             | ArcA (+) FNR (+) CpxR (-)     |
| rpoE-rseA-rseB-rseC | Sigma E and control proteins | 2.1 1.6 1.2                                                                                                                   | CpxR (-) RpoE (+)             |
| ydeH          | Conserved protein              | 2.1 2.6 2.1                                                                                                                   | CpxR (+)                      |
| yebE          | Conserved protein              | 1.7 3.2                                                                                                                       | CpxR (+)                      |
| yfdD          | PFL repair protein             | 5.9 16.4 10.7 15.5                                                                                                           | ArcA (+) FNR (+) CpxR (-)     |

* The data shown are for the first gene in the operon.

* Fold regulation (at least 2-fold at one or more time-point) is the ratio of transcript levels at the indicated time after the introduction of air to the transcript levels at t = 0 (the initial aerobic state; p ≤ 0.05).

* Relevant regulatory proteins are indicated; (−) indicates negative regulation, (+) positive regulation.

FIGURE 2. Changes in abundance of envelope stress transcripts after lowering the air supply to cultures of _E. coli_ MG1655 and inferred activities of CpxR and RpoE. A, pictorial view of changes in transcript abundance. The circles represent the CpxR and RpoE regulons as indicated. The font sizes are proportional to the fold change in transcript abundance compared with the initial aerobic state (0 min). B, activity profiles for RpoE (gray line) and CpxR (black line) generated from the transcriptomic data. The inferred activities arise from the term cm(t) in the model (Ref. 13 and supplemental materials). The error bars represent the standard deviation provided by the posterior distribution.
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The most responsive transcript during the switch to micro-aerobic conditions was ompW. The relative amount of the ompW transcript was significantly increased at all time-points tested, reaching a maximum of 28.3-fold 60 min after the air supply was lowered (pattern confirmed by RT-PCR, supplemental Fig. S1). The ompW gene encodes a β-barrel cell envelope protein that is probably involved in the transport of small molecules across the outer membrane, as well as being the receptor for colicin S4 (39). Biolog phenotyping of the ompW mutant showed metabolic defects when dipeptides (Gly-Asp, Gly-Glu, Gly-Pro) were supplied as carbon source, suggesting OmpW has a role in transporting these compounds. Homologs of E. coli OmpW have been implicated in bacterial adaptation to a range of stresses, including salinity, temperature, and O2 availability (40, 41). Inspection of the ompW promoter region revealed the presence of potential FNR (TTGATTAAAT-CAC), CpxR (GTAAAGCGGAGgtAA), and ArcA (AGTTAA-TAT; gGTgAAATTAT) binding sites (lowercase letters indicate deviations from the consensuses). The transcript start for ompW was located by 5'-RACE PCR placing the potential sites for FNR at −132.5, ArcA at −70, and −167 and CpxR at −224. In vivo transcription assays using an ompW-lacZ fusion plasmid in parent, fnr, cpxR, and arcA strains showed that ompW expression was activated by FNR, and repressed by ArcA and CpxR, under micro-aerobic conditions (supplemental Table S1).

Comparison of the Aerobic to Micro-aerobic Transition to the Anaerobic to Aerobic Transition—Although the transition described here (anaerobic to micro-aerobic conditions) is not an exact reversal of the anaerobic to aerobic transition that we described previously (20), some informative comparisons can be made.

First, comparing the responses of central metabolic transcripts suggests the identity of the key control points. Thus, here we observed decreased abundance of the PDHC transcript, increased abundance of the PFL transcript, and decreased abundance of the succinate dehydrogenase/succinyl-CoA synthetase transcript (Table 1). In the previous transition from anaerobic to aerobic conditions, these same transcripts also responded, but in the opposite direction (20). This common response confirms the importance of pyruvate metabolism and regulation of the sdh-suc operon in adapting central metabolism when O2 availability changes (26). Previously, introduction of O2 into anaerobic cultures caused the abundances of transcripts encoding anaerobic reductases and the micro-aerobic quinol oxidase (Cyd) to decrease (20). Here, upon lowering O2 availability, the opposite effects were observed. Moreover, analysis of the previous anaerobic-aerobic data to predict the activities of FNR and ArcA using the probabilistic model described above yielded activity profiles resembling those shown Fig. 1B, except that the activities are decreased during the transition rather than increased. Thus, FNR was again predicted to respond rapidly and overshoot, whereas ArcA responds more slowly with no overshoot (not shown).

Second, one of the transcriptional responses observed during the transition from anaerobic to aerobic conditions was the increased abundance of methionine biosynthetic transcripts (20). This response was transient and related to inhibition of MetE (cobalamin-independent methionine synthase) by oxidative stress (42). Interestingly, switching cultures from aerobic to micro-aerobic conditions also changed the abundances of the methionine biosynthetic transcripts, but in the opposite direction, i.e. they were less abundant under micro-aerobic conditions (not shown), consistent with an increase in the pool of active MetE when the O2 supply is restricted.

Third, the switch from anaerobic to aerobic conditions was accompanied by a transient increase in transcripts associated with the degradation of putrescine (20). Here, the switch from aerobic to micro-aerobic conditions caused a transient increase in the abundance of the speB transcript, encoding agmatine ureohydrolase, a putrescine biosynthetic enzyme. The speB transcript was most abundant (3.3-fold increase) 5 min after the air supply to the cultures was restricted, but after 60 min had almost returned to the levels present aerobically. Assuming that the increase in speB transcript abundance results in increased SpeB activity, then the rate of putrescine biosynthesis should be increased under micro-aerobic conditions. Thus, this response is consistent with the proposal that modulating intracellular putrescine concentrations is important in adaptation to changes in O2 availability, possibly through altering DNA topology (20).

In contrast to the common features of the two transitions described above, the copper management and cell envelope stress responses only seem to be associated with the switch to micro-aerobic conditions. This suggests that there are effective systems to manage these aspects in the aerobic and anaerobic steady states. Similarly, the transient OxyR-mediated oxidative stress response appears to be only associated with the anaerobic to aerobic transition (20). Thus, regardless of the direction of the transition (i.e. anaerobic to aerobic, or aerobic to micro-aerobic) central metabolism, methionine biosynthesis, and putrescine turnover appear to form part of a common core response to changes in O2 availability, but this common core is supported by additional responses to the specific challenges posed by a particular transition.

Conclusions—The ability of bacteria to respond rapidly and effectively to environmental perturbation is a distinguishing and vital aspect of their physiology. The work described here has revealed that transfer of E. coli from aerobic to micro-aerobic growth conditions causes changes in gene expression associated with central metabolism, metal ion physiology and cell envelope stress. Probabilistic modeling of the regulator activities driving transcriptome dynamics during the transition from aerobic to micro-aerobic conditions revealed fast and slow reacting components working together to mediate adaptation. Thus, the fast-reacting direct O2 sensor, FNR, and the slow-reacting indirect O2 sensor, ArcA, combine to modulate transcription of central metabolic genes. It is suggested that systems composed of rapid and slow reacting components might be a feature of other regulatory networks in which an environmental signal is perceived directly and indirectly by two or more functionally related transcription factors controlling overlapping regulons. Such systems may have evolved to allow rapid adaptation (via the fast reacting regulator), while limiting the response to short term environmental changes by mediating a
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