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Electron Acceptors and Genes: How *Escherichia coli* Adapt to Changing Environments

Hui-Chung Wu *

Department of Biotechnology, Ming Chuan University, No. 5 De-Ming Road, Gui-Shan, Taoyuan 333, Taiwan

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ABSTRACT

Escherichia coli is one of the preferred bacteria for studies on the transition and regulation of respiration pathways because of the complexity of its respiratory chains. During aerobic growth, O_2 serves as the electron acceptor for respiration. However, these organisms use a variety of compounds including nitrite (NO_2^-) and nitrate (NO_3^-) as the alternative electron acceptors during anaerobic growth. In this review, biochemical, genomic and proteomics studies were discussed to reveal the effects of alternative electron acceptors on gene expression. Transitions in gene and protein expressions were investigated. Bioinformatics database resources were also provided to investigate the integrated system biological data. Overall, the respiratory systems of *Escherichia coli* can be sketched to a great extent to adapt to the rapid and constant changes of the environments.

INTRODUCTION

Bacteria have the ability to adapt to their environmentally changes constantly and rapidly in order to survive. Enterobacteria such as *Escherichia coli* are facultative

aerobes. During aerobic growth, O_2 serves as the electron acceptor for respiration. However, these organisms use a variety of compounds including nitrite (NO_2^-) and nitrate (NO_3^-) as the alternative electron acceptors for respiration during anaerobic growth [1]. *Escherichia coli* is one of the preferred bacteria for studies on the transition and regulation of respiration pathways because the complexity of its respiratory chains. In addition, the configurations of the central metabolic pathways are also shifted constantly in order to maintain the overall balance [2]. Thus the diversity and variability of the global gene expression map is an intricate question to answer comprehensively.

As a model organism in modern biotechnology, the nucleotide sequences of many respiratory enzymes have been known and most of the enzymes have been isolated and characterized. *Escherichia coli* is one of the earliest organism to be suggested for whole genome sequencing and the complete genome sequence of *Escherichia coli* K-12 was available in 1997 [3]. Furthermore, due to the development of recent proteomics techniques, data from *Escherichia coli* proteomic studies provides a gate to reveal the global protein expression patterns [4].

In this review, biochemical, genomic and proteomics studies will be discussed to integrate a vast resource of knowledge in order to comprehensively investigate the adaptation of the respiratory systems of *Escherichia coli*.

AEROBIC AND ANAEROBIC RESPIRATORY CHAINS

As facultative aerobes, *Escherichia coli* can adapt to numerous aerobic and anaerobic respiratory systems. The synthesis of terminal respiratory enzymes is subject to hierarchical control [5] with oxygen used primary and nitrate second, following by the other acceptors [6]. Respiratory chain composition is adjusted in response to the environment in order to maintain the optimal metabolic balance [7]. The first level of hierarchical control involves response to oxygen. Aerobic cultures synthesize only basal levels of anaerobic respiratory enzymes, while adaptation to anaerobic growth is regulated by two global transcription factors, FNR (fumarate and nitrate reductase regulator) and ArcA (anaerobic respiratory control) [8]. The second level of hierarchical control, superimposed on Fnr control, involves response to nitrate, is acting through the Nar dual two component system [9, 10].

The respiratory chains of *Escherichia coli* are composed of a vast number of dehydrogenases, oxidases and reductases. The expressions of these enzymes are

regulated by these regulators. Oxygen is the preferred electron acceptor, thus the anaerobic respiratory enzymes are repressed during aerobic growth. During anaerobic growth, nitrate represses other terminal reductases, such as fumarate or DMSO reductases. Energy conservation is maximal with O_2 and lowest with fumarate [1]. Responses regarding to oxygen is regulated by a two-component regulatory system consisting of a membrane bound sensor protein ArcB and a response regulator ArcA [11]. ArcA/ArcB signal transduction system regulates gene expression in response to the redox conditions of growth. Genetic screens have lead to the identification of about 30 ArcA/ArcB controlled operons that are involved in redox metabolism. Using oligonucleotide-based microarray analysis, ArcA-P-dependent transcription profile was revealed and 55 new Arc- regulated operons were identified. The data also suggest that the Arc response pathway, which translates into a net global downscaling of gene expression, overlaps partly with the FNR regulatory network [12].

The transcription factor FNR is a cytoplasmic protein that responds to oxygen with a sensory and a regulatory DNA-binding domain. FNR is the regulator of genes required for anaerobic respiration and related pathways [13]. The current microarray data confirmed 31 of the previously characterized FNR-regulated operons. Forty four operons not previously known to be included in the FNR regulon were activated by FNR, and a further 28 operons appeared to be repressed. The FNR regulon therefore includes at least 103 and possibly as many as 115 operons [14]. The iron-sulfur cluster in FNR undergoes a reversible process that transfers between active and inactive status by the reduction -oxidation reaction [15].

Synthesis of most anaerobic respiratory pathways is subject to dual regulation by anaerobiosis and nitrate. Anaerobic induction is mediated by the FNR protein while the dual two component sensor/regulator systems control induction and repression of genes in response to nitrate/nitrite [16]. The cognate sensor proteins NARX and NARQ monitor the availability of nitrate and nitrite, and control the activity of the NARL and NARP DNA-binding proteins by phosphorylation [17, 18]. In addition, Assimilatory enzyme synthesis is induced by ammonium limitation via the NTRC protein [19] and further induced by nitrate or nitrite via the NASR protein, which may act as a transcription antiterminator [20].

EFFECTS OF ALTERNATIVE ELECTRON ACCEPTORS ON GENE EXPRESSION: GENOMIC STUDY

Recent microarray studies have identified FNR and Nar regulons comprehensively [21]. Microarray data supplemented with bioinformatic data revealed that the FNR regulon includes at least 104, and possibly as many as 115, operons, 68 of which are activated and 36 are repressed during anaerobic growth [14]. A total of 51 operons were directly or indirectly activated by NarL in response to nitrate; a further 41 operons were repressed. Global repression by the nitrate- and nitrite-responsive two- component system, NarQ-NarP, was shown for the first time. In contrast with the *frd*ABCD, *asp*A and *ans*B operons that are repressed only by NarL, the *dcu*B-*fum*B operon was among 37 operons that are repressed by NarP [21]. The transcription of genes coding for *nar*XL operon in *Escherichia coli* is controlled by ModE protein, a molybdate sensor/regulator and a second protein, MoeA. The global gene expression profile of a wild type and a *mod*E, *moe*A double mutant grown in glucose-minimal medium under anaerobic conditions were compared. Expression of 67 genes was affected by the *mod*E and *moe*A mutations [22].

Transcript microarray studies identified the *yea*R-*yoa*G operon, encoding proteins of unknown function, among genes whose transcription is induced in response to nitrate, nitrite, or nitric oxide. All known Nar-activated genes also require the oxygen- responsive Fnr transcription activator. However, further studies indicated that *yea*R-*yoa*G operon transcription does not require Fnr activation. The *yea*R-*yoa*G operon transcription is activated by phospho- NarL protein independent of the Fnr protein and the phospho-NarL protein binding site is centered at position -43.5 with respect to the transcription initiation site [23]. YeaR-yoaG operon transcription was shown to be regulated by the nitric oxide-responsive NsrR repressor [24] and mutational analyses reveal the individual contributions of the Nar and NsrR regulators to overall *yea*R-*yoa*G operon expression and document the NsrR operator centered at position -32. Thus, control of *yea*R-*yoa*G operon transcription provides an example of overlapping regulation by nitrate and nitrite, acting through the Nar regulatory system, and nitric oxide, acting through the NsrR repressor [25] and the genome- wide identification of binding sites for NsrR has been preformed [26]. In addition, enzymes involved in fumarate respiration include fumarate reductase, fumarase B, which generates fumarate from malate, and the DcuB permease for fumarate, malate, and aspartate. The transcription of the corresponding genes is activated by the DcuS-DcuR two-component system in response to fumarate or its dicarboxylate precursors. Microarray experiments that revealed two previously unknown members of the NarL regulon: the *asp*A gene encoding aspartate-ammonia lyase, which generates fumarate; and the *dcu*SR operon encoding the dicarboxylate-responsive regulatory system [27].

EFFECTS OF ALTERNATIVE ELECTRON ACCEPTORS ON PROTEIN EXPRESSION: PROTEOMIC STUDY

The *Escherichia coli* proteome has been extensively studied and is well defined in terms of biochemical, biological, and biotechnological data [4]. It has been shown that the expression of catabolic enzymes and periplasmic proteins is regulated by pH and the modes of pH regulation were revealed under anaerobiosis using proteomic two-dimensional gels approach. A total of 32 proteins from anaerobic cultures show pH-dependent expression, and four of these proteins (DsbA, TnaA, GatY, and HdeA) showed pH regulation in aerated cultures. The levels of 19 proteins were elevated at the high pH; these proteins included metabolic enzymes, periplasmic proteins, and stress proteins. On the other hand, 13 other proteins were induced by acid; these proteins included metabolic enzymes, periplasmic proteins and redox enzymes [28]. A proteomic analysis of *Escherichia coli* in which 3,199 protein forms were detected, and of those 2,160 were annotated and assigned to the cytosol, periplasm, inner membrane, and outer membrane by biochemical fractionation followed by two-dimensional gel electrophoresis and tandem mass spectrometry [29]. Relatively, *Escherichia coli* proteome is one of the most characterized bank and a reference protein map of *E. coli* obtained with immobilized pH gradients (IPG) and available in a SWISS-2DPAGE format [30]. Differential expression data to investigate proteins under anaerobic conditions with or in the absence of nitrate can also be found [29].

DATABASE RESOURCE

Owing to its model role in modern biology, *Escherichia coli* are the best-studied organism for the respiratory pathways. Many bioinformatic databases have already existed, such as NCBI, ExPASy and so on. A comprehensive view of *Escherichia coli* biology can be found at the EcoCyc [31]. EcoCyc is a member of a larger collection of Pathway/ Genome Databases (PGDBs) called BioCyc available at http://Biocyc.org/. The EcoCyc database (http://EcoCyc.org/) provides comprehensive source of *Escherichia coli* K12 including operons, genetic networks, transcription factor binding sites, metabolic pathways, functionally related genes, protein complexes and protein–ligand interactions. The information regarding to the respiratory systems of *Escherichia coli* can be obtained with the integrated system biological data.

CONCLUDING REMARKS

In summary, *Escherichia coli* are facultative aerobes and therefore can adapt to a number of respiratory pathways constantly and rapidly. During aerobic growth, O_2 serves as the electron acceptor for respiration. For the duration of anaerobic growth, these organisms use a variety of compounds including nitrite (NO_2^-) and nitrate (NO_3^-) as the alternative electron acceptors for respiration. The key enzymes and regulators have been identified extensively, where adaptation to anaerobic growth is regulated by two global transcription factors, FNR and ArcA. In addition, adaptation to nitrate and alternative electron acceptors is acting through the Nar dual two component system. Owning to the rapid developments of genomic and proteomics techniques, the respiratory systems of *Escherichia coli* can be investigated globally with hundreds and thousands genes investigated simultaneously. In this review, recent studies to explore the regulations of the respiratory chains in *Escherichia coli* were discussed with emphasis in genomic and proteomics updates. By integrating the system biological data, the adaptation of the respiratory systems of *Escherichia coli* can be sketched to a great extent.

FOOTNOTES

*Corresponding author, e-mail: joannawu@mail.mcu.edu.tw

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Ming Chuan-Health Tech Jounal, 2010, Vol. 1, No. 1, e1