J Ind Microbiol Biotechnol (2017) 44:647–658
DOI 10.1007/s10295-016-1851-6

METABOLIC ENGINEERING AND SYNTHETIC BIOLOGY - REVIEW

Strategies for manipulation of oxygen utilization by the electron transfer chain in microbes for metabolic engineering purposes

George N. Bennett[1,3] · **Ka-Yiu San**[2,3]

Received: 26 August 2016 / Accepted: 6 October 2016 / Published online: 31 October 2016

Abstract Microaerobic growth is of importance in ecological niches, pathogenic infections and industrial production of chemicals. The use of low levels of oxygen enables the cell to gain energy and grow more robustly in the presence of a carbon source that can be oxidized and provide electrons to the respiratory chain in the membrane. A considerable amount of information is available on the genes and proteins involved in respiratory growth and the regulation of genes involved in aerobic and anaerobic metabolism. The dependence of regulation on sensing systems that respond to reduced quinones (e.g. ArcB) or oxygen levels that affect labile redox components of transcription regulators (Fnr) are key in understanding the regulation. Manipulation of the amount of respiration can be difficult to control in dense cultures or inadequately mixed reactors leading to inhomogeneous cultures that may have lower than optimal performance. Efforts to control respiration through genetic means have been reported and address mutations affecting components of the electron transport chain. In a recent report completion for intermediates of the ubiquinone biosynthetic pathway was used to dial the level of respiration vs lactate formation in an aerobically grown *E. coli* culture.

✉ Ka-Yiu San
ksan@rice.edu

[1] Department of BioSciences, Rice University, Houston, TX 77005, USA

[2] Department of Bioengineering, Rice University, Houston, TX 77005, USA

[3] Department of Chemical and Biomolecular Engineering, Rice University, Houston, TX 77005, USA

Keywords *Escherichia coli* · Electron transfer chain · Quinone · Metabolome · Oxygen · Regulation · Enzyme · Competition

Introduction

Understanding microaerobic growth is important in appreciating interactions in microbial communities, in the infection process of certain pathogens, and industrially in how we might better manipulate commercial large-scale cultures for efficient and reliable product formation. A number of articles have examined effects of microaerobic conditions on organisms from ecological and health perspectives [63, 79, 112], photosynthetic microbe biology [22, 132] and in pathogen invasion [108, 113] and infection specifically by Neisseria [4], and Campylobacter [73]. Many large-scale industrial microbiological processes operate under partial aerobic conditions. For example, the production of PHB [70, 116, 131], polysaccharide based biopolymers [2, 16, 43], lycopene and carotenoids [92], IPP-terpene processes for pharmaceuticals [35, 99, 114], and alcoholic biofuels [91, 117] [9, 15] have been discussed in reference to limited oxygen culture conditions. Other examples of use of microaerobic production conditions have appeared for lactate [39, 101], acetoin [28], succinate [87], 2,3-butanediol [102], and H_2S removal [129]. The metabolic reprogramming occurring under microaerobic conditions has been considered along with the effect of several regulators FNR, ArcAB, NarL, NarP [140]. The screening of strains for high oxygen utilization has been emphasized for identification of industrial production organisms [176].

In industrial practice, to produce high levels of product, high cell density is required that further taxes the ability to properly aerate the culture. Inadequate mixing in large

Table 1 Factors influencing respiration

Regulators of anaerobic and aerobic active metabolic genes	Function
ArcAB	Senses redox state of quinone in membrane and affects gene expression of aerobic and anaerobic metabolic genes
Fnr	Becomes active at low oxygen and enhances anaerobic metabolism
FnrS, arcZ, FrsA, Dan	Modulators of the aerobic anaerobic transition through effects on gene regulation
Proteins involved in the function of oxidizing NADH	
Cytochrome oxidase genes: *cydAB*, *cyoABCD*, and *cbdAB*	Perform the connection between the reduced membrane protein and oxygen in the ETS
Nuo system	Provide a means of oxidizing NADH and feeding the ETS
NADH oxidase	Provides a direct means of oxidizing NADH
Electron carriers	
Ubiquinone	Acts as the major electron carrier in the membrane to the cytochrome oxidases under aerobic conditions
NADH	The main soluble electron carrier in *E. coli* coupling with many cell metabolic oxo-reduction reactions

reactors coupled with limiting oxygen solubility and gas transfer obstacles are serious problems affecting reliability and optimal productivity. In these large-scale processes inefficient production by a fraction of cells can have serious economic consequences as the non-productive oxygen limited cells not only do not produce the desired product in the normal amount, but also their metabolic by-products can affect the other cells in the bioreactor, limiting overall performance. Efforts to control such processes by reactor design, gas transfer systems, and optimized electronic sensor-response systems have made technological advances for bioprocessing. However, the fundamental question of the physiology of microbial host and genetic modification of the host to optimize oxygen utilization has been less completely addressed.

There is a considerable background on factors affecting regulation of respiration. Too much respiration and oxidation of carbon substrates, while being beneficial for cell energetics and for growth rate, lead to loss of carbon that does not go into product, especially if a reduced product is desired. Many reduced products of commercial interest, such as fuel molecules, are compounds more reduced than glucose, and many chemical intermediates for pharmaceuticals, lactate or monomers for making polymers, fatty acids, etc. also require reduction reactions and similarly optimal formation of these molecules requires limiting oxidation of the feedstock for a high carbon atom yield process.

Respiration pathways

Oxygen is an effective electronic acceptor and can provide a significantly higher ATP/glucose yield (more than 30 ATP per glucose under aerobic conditions vs only 2 ATP from the glycolysis pathway under anaerobic conditions). Aerobic cultures are, therefore, in general more robust than their anaerobic counterpart. However, since NADH is being consumed in the electron transfer chain (ETC), the intracellular NADH/NAD^+ ratio has been shown to decrease significantly with increasing culture dissolved oxygen levels [139]. Hence, these opposing trends impose conflicting demands: a robust culture for rapid cell growth to achieve high biomass and high cell energetics under aerobic metabolism, and the opposing demand for NADH in product formation most readily attained under anaerobic conditions. Microaerobic conditions have thus been shown experimentally and theoretically to improve performance of a number of bioproduction systems. However, it is difficult to maintain a set dissolved oxygen level in a large-scale production environment due to incomplete mixing and balance with the rate of oxygen uptake by culture.

As part of its ability to adapt to different growth conditions, *E. coli* alters the composition of its respiratory system [159]. Important factors influencing aerobic respiration are listed in Table 1. The three types of respiratory components are (1) dehydrogenases, which carry out the oxidation of organic substrates and feed electrons into the mobile quinone pool, (2) quinones, which deliver reducing equivalents to the terminal oxidoreductases, and (3) oxidoreductases, which reduce the terminal electron acceptors [44]. The ETC of *E. coli* is composed of membrane-anchored dehydrogenases that reduce the quinone pool (ubiquinone-8, Q8) under respiratory conditions. Of these, the nuo and ndh pathways are most important in aerobic conditions. The quinone redox state is sensed by the ArcB protein and relayed through phosphorylation of the transcriptional regulator ArcA, to affect expression of genes of the TCA cycle and the electron transport chain; these levels of functional components are then adjusted to modify the cell's respiration vs fermentative metabolism in an

aerobic–anaerobic transition. The amount of each component is strictly regulated to optimize the respiratory chain according to the substrates present and the physiological needs of the cell. One important function of the respiratory chain is the maintenance of redox balance and the regeneration of NAD^+ from NADH. Under aerobic growth *E. coli* normally makes two different NADH dehydrogenases, NAD I and NAD II, and two different terminal oxidases, cytochrome bo3 and cytochrome bd. The electron flux through these enzymes is dependent on the concentrations of the enzyme in the membrane, the NADH, quinone, and oxygen concentrations, and the steady-state characteristics of the enzymes (i.e. V_{max} and K_m values for NADH, quinone and oxygen).

E. coli cells regenerate NAD^+ and generate proton motive force for ATP production through the respiratory chain. One way to reduce the activity of ETC and thus the amount of oxygen used is reducing the cytochrome protein levels [61, 78, 127, 128] or affecting assembly of the protein–membrane complex [95] and both of these ways affect the proteins responsible for the utilization of NADH. Another way is to control the level of quinone electron carrier involved in the transfer of electrons from NADH to essential membrane proteins during the process by inactivating its biosynthetic pathway [147] and then adding different amounts of an analog of quinone back to the culture of cells that are unable to synthesize the quinone, such as coenzyme Q1 [172] and noting the change in metabolites formed.

Regulation of proteins involved in the aerobic–anaerobic transition

The regulation of the aerobic–anaerobic shift in *E. coli* has been extensively studied. *E. coli* possesses a number of sensing/regulation systems for the response to availability of oxygen [3, 12, 30, 53, 65, 72, 90, 96, 122, 133, 134, 136, 157, 158] and channels electrons from donor to terminal acceptors such that the overall redox potential difference is maximized for any given growth condition. The adaptive responses are coordinated by a group of global regulators, which includes Fnr (fumarate, nitrate reduction) protein, and the two-component Arc (aerobic respiration control) system. With the initial onset of anaerobiosis, ArcA is activated, and if these conditions persist or become more anaerobic, Fnr is activated leading to the upregulation of ArcA amplifying its effect [72]. The oxygen levels (percent oxygen) that gave rise to half-maximal synthesis were reported as 0.02–0.04% for ethanol, acetate, and succinate, and 0.1% for formate [12]. The $pO_{0.5}$, which is defined as the pO_2 value resulting in a half-maximal expression, for expression of the *adhE* gene encoding alcohol dehydrogenase was ~0.08% oxygen [12] and for expression of cytochrome oxidases was measured [154] for *cyd* (maximal at 1–1.4%) and *cyo* shut-off at 1–4%. The global gene expression effects of Fnr and ArcA have been reported [11, 133, 134] with the Fnr transition occurring at 0.1–2% and Arc at 1–4%. The Arc system is a two-component regulatory system composed of ArcA, the cytosolic response regulator, and ArcB, the transmembrane histidine kinase sensor. ArcB is activated during the transition from aerobic to microanaerobic growth [66, 67] in response to the redox composition of the quinone pool [46] including menaquinone or dimethylmenaquinone [1, 13, 141] whose synthesis does not require oxygen [94, 103, 143, 159]. ArcB undergoes autophosphorylation, and the ~P group is transferred to ArcA by a His → Asp → His → Asp phosphorelay [45, 47, 84]. Consequently, the increased level of phosphorylated ArcA represses the synthesis of the citric acid cycle enzymes, while it activates the expression of cytochrome d oxidase and enzymes involved in fermentative metabolism [90, 96, 158]. The Fnr protein, [157, 160] is a transcription factor that coordinates the switch between aerobic and anaerobic metabolism at low oxygen levels. Fnr contains a Fe-S cluster that serves as a redox sensor. In the presence of oxygen Fnr is converted to a non-DNA binding monomer form [11, 53]. The Fnr system induces the expression of genes that permit anaerobically growing *E. coli* to transfer electrons to alternative terminal acceptors [51, 90]. Moreover, active Fnr elevates expression of *arcA* in anaerobic cells. Fnr also affects a number of genes in *E. coli* whose function has not been determined [75].

The concentration of certain anaerobic metabolites also affects ArcB function [96]. A mutant of Fnr that is active aerobically [68] has been used in metabolic experiments of the aerobic–anaerobic transition [137–139] and these studies suggested the recycling of Fnr to the active form was an important aspect. The Fnr protein cycle has been examined theoretically [152, 153] to prepare a more complete kinetic model of Fnr activity that included assembly of the iron-sulfur cluster and degradation by ClpXP protease [153]. The simulation of *E. coli* response to sudden oxygen starvation has been reported with software for analyzing and modeling the process [135].

A kinetic model of oxygen regulation of cytochrome production has been developed [124], and the metabolic flux analysis of various *arc*, *fnr* or other strains using C-13 labeling technique has been reported [118, 173, 174]. The new findings of additional factors affecting the anaerobic–aerobic transition such as small RNAs [48, 49, 97] add complexity to the models of the transition. For example, ArcZ directly represses *arcB*, and is itself repressed by the ArcBA system, providing a negative feedback loop [97]. Another example is FnrS, a highly conserved, anaerobically induced small sRNA, whose expression is strictly dependent on Fnr and negatively regulates many genes encoding enzymes

with aerobic functions [19, 37]. These studies add to the wide impact of processes regulated by the Arc and Fnr systems under microaerobic conditions [13, 19, 37, 125]. The discovery of new proteins that influence components of the transition, for example, the SixA phosphatase [55, 100, 119], FrsA (fermentation protein) [81], and the major anaerobic nucleoid protein, Dan, that acts in DNA filament formation [89, 151] indicate there are further characters to consider in the aerobic–anaerobic transition in more detail.

Correlating the metabolic consequences of low oxygen with gene expression studies

The effects of low oxygen on gene expression and cell metabolism have been studied using C-13 labeling and flux analysis [144]. Studies have examined dynamics [34] and correlated models with experiments [23]. The general foundation concerning genes, regulators, and physiological implications of low oxygen have been reviewed [13, 17, 54, 64, 154]. As oxygen levels are reduced the cell will shift from the use of the Cyo pathway to the Cyd pathway, then at lower oxygen levels the fermentative pathway engages, oxygen using pathways are shut down and NADH formed in glycolytic processes is recycled by forming reduced products from pyruvate, e.g. lactate and ethanol. The change in metabolite pattern in response to availability of oxygen also modulates gene expression as well as enzyme activity. The flow through the citric acid cycle is inhibited by product accumulation. Acetyl-CoA is produced in oxygen-rich environments by pyruvate dehydrogenase complex (Pdh) or anaerobically by pyruvate formate lyase (Pfl). Pdh is inhibited by ATP, acetyl-CoA, and NADH [41, 50, 76]. Active Pfl is a radical form protein inactivated by oxygen [85] but the YfiD protein can reactivate Pfl [162, 169]. Reduced flux of pyruvate through Pfl and Pdh results in pyruvate accumulation that activates lactate dehydrogenase (Ldh) [164]. These soluble metabolites exhibit effects on gene expression [77] and can affect physiological properties of surrounding cells. Useful recent models of the central metabolic pathway [27, 74], the response of the Fnr system [152, 153], and the integration of "omic" and signal transduction data [31] have been published and serve as a framework in further modeling the transition.

The electron carrier quinones of the electron transfer chain

The lipid-soluble quinones that carry electrons within the membrane between electron input protein complexes and the cytochrome oxidases for reaction with the electron acceptor (oxygen) are generally ubiquinone with an isoprene tail of varying length in different organisms and this biochemistry and the methods for production of Coenzyme Q or menaquinone (vitamin K) for health have been reviewed [5, 6, 29, 69, 80, 103, 104, 146, 163]. Under more anaerobic conditions or when using a different electron acceptor, menaquinone or dimethylmenaquinone acts as the carrier [142, 166]. The composition and level of the quinone carrier pool is altered under differing conditions of electron acceptors [143, 165].

The biosynthetic pathways of the quinone carriers have been determined and the enzymes and corresponding genes of *E. coli* have been identified. Studies of the growth of various mutants and the levels of ubiquinone have been reported. There is a low amount of ubiquinone in the cell under normal aerobic growth conditions. However, this low concentration is more than sufficient since mutations resulting in a moderately reduced level do not have drastic effects on cell growth physiology but grow more slowly [115] and have hypersensitivity to thiols [170]. For example, ispA mutants disrupted for farnesyl diphosphate synthase have lower levels of ubiquinone-8 and menaquinone-8 (less than 13 and 18%), respectively [42]. UbiX mutants (an alternative to UbiD activity) have lower ubiquinone levels [52, 171]. The ability of ubiquinone-8 to serve in the electron transfer chain with type II dehydrogenases was somewhat limited in membranes and cells that did not contain phosphatidylethanolamine in the phospholipid [107].

Genetic approaches to control respiration and oxygen utilization

Recent work on regulating respiration and generating altered *E. coli* that could form lactate or other reduced compounds under aerobic conditions has progressed based on the literature of genes required for respiration and newly appreciated genes that have a role in redox transfer. This avenue was advanced by Palsson's group in articles demonstrating aerobic fermentation properties on *E. coli* with several mutations [127, 128]. In that work the terminal cytochrome oxidase genes (*cydAB*, *cyoABCD*, and *cbdAB*) were inactivated and subsequent selection allowed a strain to be obtained that could carry out mixed acid or primarily lactate production (0.8 g/g lactate from glucose) under aerobic conditions. Additional removal of a quinol monooxygenase gene (*ygiN*) led to activation of ArcA aerobically. This strain formed D-lactate as a sole by-product under both oxic and anoxic conditions. The quinone pool changed from ubiquinones in normal cells to menaquinones in the mutant strain [128]. This physiological situation led to activation of the ArcB/ArcA system and altered the metabolic flux pattern with the flux through the tricarboxylic acid (TCA) cycle being greatly reduced, while glycolysis and formation of oxaloacetate were enhanced. The finding that transcriptomic results could be correlated with the

in vivo function was encouraging for future gene manipulation strategies in the redox arena. In studies of *E. coli* mutants with deletions of three cytochrome terminal oxidases the results showed a fermentative metabolic pattern in the presence of oxygen [14]. The electron flux through cytochrome bd-II oxidase did not form a proton motive force for ATP generation and cells then conducted ATP synthesis only by substrate level phosphorylation reactions. Thus the P/O ratios can vary severalfold depending on the electron flux distribution through the respiratory chain. An interesting hypothesis based on a genome-scale metabolic model of *E. coli* was proposed [175]. The authors proposed that bacterial cells managed the protein composition of the cytoplasmic membrane for optimal ATP production under the growth condition by regulating the production of ATP by either the coupled ETC or substrate-level reactions and that membrane occupancy and total allowable protein level in the membrane may constrain cell metabolism, phenotypic properties and oxygen-energy physiology. The study of gene regulation and coordination of nitrogen and carbon metabolism by analysis of transcript levels of metabolic pathway genes and metabolic gene regulators in *E. coli* with mutations in cytochrome oxidases, and regulatory genes, *fnr*, and *fur* and others, also demonstrated the value of transcriptomics in metabolic analysis [82, 83]. The examination of the coordination of metabolic consequences of such genetic changes suggested an interconnection between cAMP and PII-Nitrogen regulatory systems and illustrates the connections of more complex networks that should be taken into account in practical applications related to metabolic engineering and defining optimal culture conditions.

The idea of control of respiration and of avoiding excessive loss of carbon to the formation of undesired products such as CO_2 has been taken to the industrial organisms, *Corynebacterium glutamicum* [78] and *Zymomonas mobilis* [60]. A *C. glutamicum* strain that had a completely inactivated aerobic respiratory chain (*cydAB qcr*) was able to grow aerobically in complex medium but had a 70% reduced biomass yield and could also grow in glucose minimal medium after supplementation with peptone. The modified *C. glutamicum* strain displayed a fermentative metabolism with L-lactate as the major metabolite and succinate and acetate formed in lesser quantity. The observation that phosphofructose kinase overexpression is critical for high production of lactate in *C. glutamicum* under oxygen deprivation has been reported [155]. The use of the normally aerobic organism, *C. glutamicum,* under oxygen deprivation has been examined for high yield production of several commercial products [56, 57, 71, 106, 120, 121]. In the case of *Z. mobilis*, mutation of genes leading to respiratory deficiency generated strains with higher ethanol fermentation under aerobic conditions. The strains were also more thermotolerant and it was considered that the strains benefited from the lower amount of reactive oxygen species and reduced stress associated with oxygen respiration.

The use of oxygen in the electron transport chain has been studied in *E. coli* and many organisms. This membrane system contains various cytochromes and electron carriers, such as the quinone, ubiquinone. One way to try to control the extent of electron transfer chain (ETC) activity and thus the amount of oxygen used would be to control the cytochrome protein levels by controlling their expression [10, 109, 110]. This strategy exists in the native *cyo* and *cyd* systems that respond to different levels of oxygen [17, 109, 154]. Another viewpoint is to consider the level of quinone [5]; Zhu et al. [172] have shown by knockout of the biosynthesis pathway and adding small amounts of ubiquinone back to the culture, respiration could be controlled. Another approach is to modify the biosynthesis of the quinone electron carrier by altering expression of that operon encoding the enzymes of the biosynthetic pathway. Some of these alternatives have been explored previously; however, it is experimentally difficult to control the exact level of a partially-on system by these means, and generally it was found that cells behaved either as unaffected wild type or were completely inactive for the pathway, enhancing interest in other ways to finely control levels of such key molecules by genetic control of the network (Bennett and San, unpublished data). In general, control can be difficult since the level of the factor may be very low, for example, the quinone pool is only about 1 nmol/mg DCW [38, 52, 170, 171] so changes in expression of the operon encoding the biosynthetic pathway for the quinone can overshoot or undershoot the transition threshold level and make the ETC always off or always on.

The concept of manipulation of a key cofactor to control a large flux pathway

In cell metabolism, it is desired to control a large flux using a controller that can be regulated at an appropriate level, either at a defined fixed level or at a feedback controlled response level. At the genetic level, synthetic biology approaches of "gene circuits" have allowed control of cell responses [58, 59, 98, 130, 148, 150] by modifications in promoter strength [20, 33, 105], subjecting expression to exogenous parameters such as inducer or light [86, 149] or cell formed substances in feedback loops [21, 26, 36, 62, 93] using repressors and antirepressors [24], activators and layered circuits [111], inverters [40, 123], or RNA responsive elements that act primarily at the level of transcription or translation [7, 8, 18, 25, 32, 88, 126, 145] which affect the level of the appropriate enzyme(s) of a biosynthetic pathway.

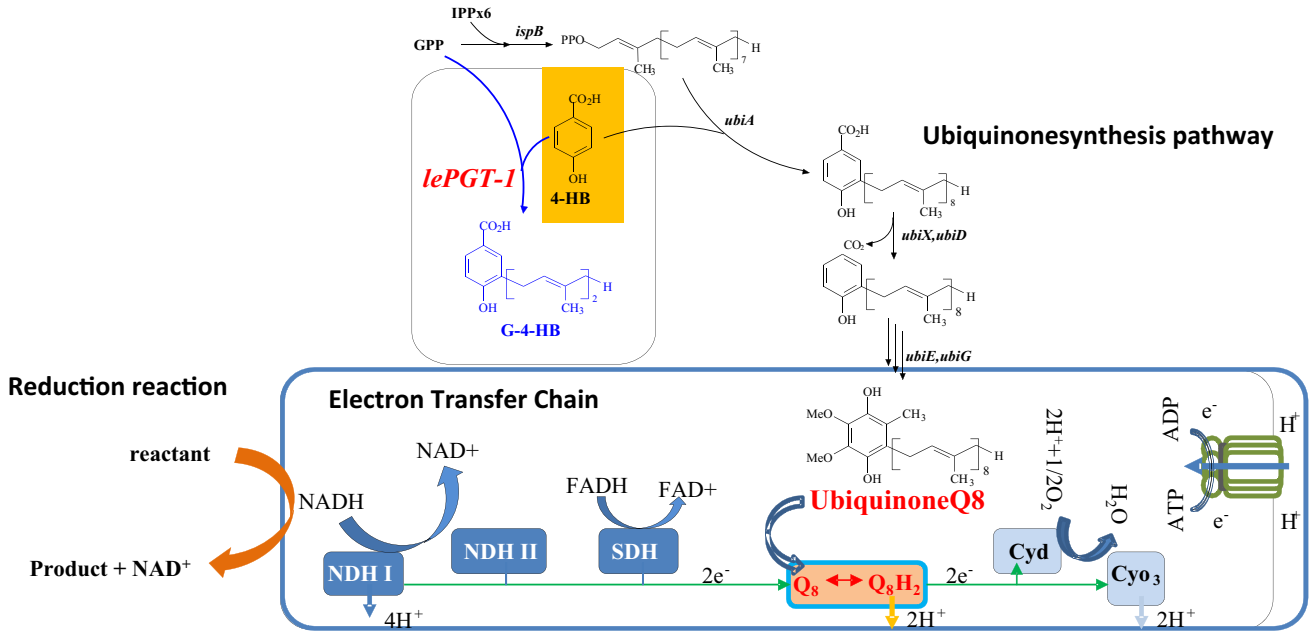

Fig. 1 A schematic showing the interplay/competition for NADH between the electron transfer chain (ETC) and a reduction reaction leading to reduced product formation. The metabolic transistor approach to control the ETC activity through Q8 manipulations is also shown. The intracellular ubiquinone Q8 concentration can be adjusted by dialing lePGT-1 expression up and down. Higher lePGT-1 expression increases the drainage of two key Q8 biosynthetic precursors, GPP and 4-HB, leading to a lower intracellular Q8 concentration. In addition, controlling the level of intracellular Q8 has a direct influence on the ETC activity. Lower the ubiquinone Q8 concentration will reduce ETC activity and increase NADH availability. Increased NADH availability will provide more reducing equivalence for reduced product formation. *Gpp* geranyl pyrophosphate, *4-HB* 4-hydrobenzoate, *G-4-HB* geranyl-4-hydrobenzoate, *lePGT-1* geranyl diphosphate:4-hydroxybenzoate geranyltransferase from *Lithospermum erythrorhizon*

An additional strategy to finely control a large metabolic flux by using a "metabolic transistor" approach has been proposed and its implementation feasibility has been examined [168]. In this approach a small change in the level or availability of a key participant in generating the large flux is controlled and the change in this basal level, considered as a base current in a transistor, has a large effect on the flux through the major pathway analogous to the change in current in an electrical transistor [156, 161]. There are a number of ways in which the level of a small molecule in the cell can be manipulated. Among these are the direct alteration of the expression of the biosynthetic pathway via promoter or translation control; another means to affect the level could be to degrade, excrete or otherwise bind the small molecule so it cannot participate in the reaction where it is required for the major flux.

In the "metabolic transistor" approach, the precursors leading to the biosynthetic pathway of the small molecule were being manipulated by effective partitioning of these precursor intermediates at the introduced node. This precursor control approach is particularly effective for a pathway network that consists of irreversible or close to irreversible reactions since the first step of the pathway network exerts the most control on its metabolic flux according to metabolic control theory. Wu et al. [168] has successfully demonstrated the control the ETC in wild-type *E. coli* by fine-turning the expression of geranyl diphosphate:4-hydroxybenzoate geranyltransferase from *Lithospermum erythrorhizon* (le*PGT*-1). The reaction catalyzed by lePGT-1 plays as a competing pathway on the substrates of the ubiquinone-8 (Q8) synthesis pathway, namely at IPP and 4-HB, and introduction of this new node is effective in affecting the flow through the Q8 biosynthetic pathway and subsequently the level of Q8. The Q8, which serves to deliver reducing equivalents between electron donors, such as NADH dehydrogenase (NDH), succinate dehydrogenase (SDH), and terminal electron acceptors, such as cytochrome oxidases or reductases, is an essential element under aerobic respiratory conditions [6] (Fig. 1). Hence, controlling the level of intracellular Q8 has a direct influence on the ETC activity.

Using this approach, Wu et al. [167, 168] showed that it is possible to control the activity of the electron transfer chain in a tunable manner and manipulate the production of reduced products while limiting consumption of oxygen to a defined amount even under fully aerobic conditions. The intracellular Q8 concentrations showed a graded response to the lePGT-1 induction levels (IPTG concentrations).

Furthermore, the authors have shown that it is possible to achieve a maximum theoretical yield of lactate production under fully aerobic conditions and minimize the "carbon burnt" (carbon lost as carbon dioxide) by limiting the ETC activity via lowering the intracellular Q8 concentrations.

Conclusions and future prospects

The complexity of respiratory metabolism and its control has been explored through biochemical and genetic experiments. Routes for the more precise control of oxygen utilization under conditions providing microaerobic culture conditions have been proposed and demonstrated. With the advent of synthetic biology and the ability to test numerous regulatory designs, particular applications useful to industrial processes that allow more reliable and controllable and optimized operations of large-scale cultures are likely to be implemented. The potential application of a "metabolic transistor" approach to control respiration, such as situations where biosynthesis or availability of a limiting factor is crucial for a biological process, is appealing. With further study of the variety of respiratory systems existing in wide diversity of microbes, the understanding of the controls that make an organism well suited to an ecological niche will become more apparent.

Acknowledgements The work was supported in part by a grant from the National Institutes of Health (NIH GM090152).

References

1. Alvarez AF, Rodriguez C, Georgellis D (2013) Ubiquinone and menaquinone electron carriers represent the yin and yang in the redox regulation of the ArcB sensor kinase. J Bacteriol 195:3054–3061. doi:10.1128/JB.00406-13
2. Amanullah A, Tuttiett B, Nienow AW (1998) Agitator speed and dissolved oxygen effects in xanthan fermentations. Biotechnol Bioeng 57:198–210
3. Arras T, Schirawski J, Unden G (1998) Availability of O2 as a substrate in the cytoplasm of bacteria under aerobic and microaerobic conditions. J Bacteriol 180:2133–2136
4. Atack JM, Ibranovic I, Ong CL, Djoko KY, Chen NH, Vanden Hoven R, Jennings MP, Edwards JL, McEwan AG (2014) A role for lactate dehydrogenases in the survival of *Neisseria gonorrhoeae* in human polymorphonuclear leukocytes and cervical epithelial cells. J Infect Dis 210:1311–1318. doi:10.1093/infdis/jiu230
5. Au DC, Green GN, Gennis RB (1984) Role of quinones in the branch of the *Escherichia coli* respiratory chain that terminates in cytochrome o. J Bacteriol 157:122–125
6. Aussel L, Pierrel F, Loiseau L, Lombard M, Fontecave M, Barras F (2014) Biosynthesis and physiology of coenzyme Q in bacteria. Biochim Biophys Acta 1837:1004–1011. doi:10.1016/j.bbabio.2014.01.015
7. Babiskin AH, Smolke CD (2011) A synthetic library of RNA control modules for predictable tuning of gene expression in yeast. Mol Syst Biol 7:471. doi:10.1038/msb.2011.4
8. Babiskin AH, Smolke CD (2011) Synthetic RNA modules for fine-tuning gene expression levels in yeast by modulating RNase III activity. Nucleic Acids Res 39:8651–8664. doi:10.1093/nar/gkr445
9. Baez A, Cho KM, Liao JC (2011) High-flux isobutanol production using engineered Escherichia coli: a bioreactor study with in situ product removal. Appl Microbiol Biotechnol 90:1681–1690. doi:10.1007/s00253-011-3173-y
10. Bansal K, Yang K, Nistala GJ, Gennis RB, Bhalerao KD (2010) A positive feedback-based gene circuit to increase the production of a membrane protein. J Biol Eng 4:6. doi:10.1186/1754-1611-4-6
11. Becker S, Holighaus G, Gabrielczyk T, Unden G (1996) O2 as the regulatory signal for FNR-dependent gene regulation in *Escherichia coli*. J Bacteriol 178:4515–4521
12. Becker S, Vlad D, Schuster S, Pfeiffer P, Unden G (1997) Regulatory O2 tensions for the synthesis of fermentation products in *Escherichia coli* and relation to aerobic respiration. Arch Microbiol 168:290–296
13. Bekker M, Alexeeva S, Laan W, Sawers G, Teixeira de Mattos J, Hellingwerf K (2010) The ArcBA two-component system of *Escherichia coli* is regulated by the redox state of both the ubiquinone and the menaquinone pool. J Bacteriol 192:746–754. doi:10.1128/JB.01156-09
14. Bekker M, de Vries S, Ter Beek A, Hellingwerf KJ, de Mattos MJ (2009) Respiration of *Escherichia coli* can be fully uncoupled via the nonelectrogenic terminal cytochrome bd-II oxidase. J Bacteriol 191:5510–5517. doi:10.1128/JB.00562-09
15. Blombach B, Riester T, Wieschalka S, Ziert C, Youn JW, Wendisch VF, Eikmanns BJ (2011) *Corynebacterium glutamicum* tailored for efficient isobutanol production. Appl Environ Microbiol 77:3300–3310. doi:10.1128/AEM.02972-10
16. Borges CD, da Moreira AS, Vendruscolo CT, Ayub MA (2008) Influence of agitation and aeration in xanthan production by *Xanthomonas campestris* pv pruni strain 101. Rev Argent Microbiol 40:81–85
17. Borisov VB, Gennis RB, Hemp J, Verkhovsky MI (2011) The cytochrome bd respiratory oxygen reductases. Biochim Biophys Acta 1807:1398–1413. doi:10.1016/j.bbabio.2011.06.016
18. Boyle PM, Silver PA (2012) Parts plus pipes: synthetic biology approaches to metabolic engineering. Metab Eng 14:223–232. doi:10.1016/j.ymben.2011.10.003
19. Boysen A, Moller-Jensen J, Kallipolitis B, Valentin-Hansen P, Overgaard M (2010) Translational regulation of gene expression by an anaerobically induced small non-coding RNA in *Escherichia coli*. J Biol Chem 285:10690–10702
20. Brewster RC, Jones DL, Phillips R (2012) Tuning promoter strength through RNA polymerase binding site design in *Escherichia coli*. PLoS Comput Biol 8:e1002811. doi:10.1371/journal.pcbi.1002811
21. Bulter T, Lee SG, Wong WW, Fung E, Connor MR, Liao JC (2004) Design of artificial cell-cell communication using gene and metabolic networks. Proc Natl Acad Sci USA 101:2299–2304
22. Carius L, Hadicke O, Grammel H (2013) Stepwise reduction of the culture redox potential allows the analysis of microaerobic metabolism and photosynthetic membrane synthesis in *Rhodospirillum rubrum*. Biotechnol Bioeng 110:573–585. doi:10.1002/bit.24734
23. Carlson R, Srienc F (2004) Fundamental *Escherichia coli* biochemical pathways for biomass and energy production: creation of overall flux states. Biotechnol Bioeng 86:149–162
24. Chai Y, Kolter R, Losick R (2009) Paralogous antirepressors acting on the master regulator for biofilm formation in *Bacillus subtilis*. Mol Microbiol 74:876–887. doi:10.1111/j.1365-2958.2009.06900.x

25. Chang AL, Wolf JJ, Smolke CD (2012) Synthetic RNA switches as a tool for temporal and spatial control over gene expression. Curr Opin Biotechnol 23:679–688. doi:10.1016/j.copbio.2012.01.005
26. Chang DE, Leung S, Atkinson MR, Reifler A, Forger D, Ninfa AJ (2010) Building biological memory by linking positive feedback loops. Proc Natl Acad Sci USA 107:175–180. doi:10.1073/pnas.0908314107
27. Chassagnole C, Noisommit-Rizzi N, Schmid JW, Mauch K, Reuss M (2002) Dynamic modeling of the central carbon metabolism of *Escherichia coli*. Biotechnol Bioeng 79:53–73. doi:10.1002/bit.10288
28. Chen T, Liu WX, Fu J, Zhang B, Tang YJ (2013) Engineering *Bacillus subtilis* for acetoin production from glucose and xylose mixtures. J Biotechnol 168:499–505. doi:10.1016/j.jbiotec.2013.09.020
29. Cluis CP, Pinel D, Martin VJ (2012) The production of coenzyme Q10 in microorganisms. Sub-Cellular Biochem 64:303–326. doi:10.1007/978-94-007-5055-5_15
30. Cotter PA, Melville SB, Albrecht JA, Gunsalus RP (1997) Aerobic regulation of cytochrome d oxidase (*cydAB*) operon expression in *Escherichia coli*: roles of Fnr and ArcA in repression and activation. Mol Microbiol 25:605–615
31. Covert MW, Xiao N, Chen TJ, Karr JR (2008) Integrating metabolic, transcriptional regulatory and signal transduction models in *Escherichia coli*. Bioinformatics 24:2044–2050. doi:10.1093/bioinformatics/btn352
32. Culler SJ, Hoff KG, Smolke CD (2010) Reprogramming cellular behavior with RNA controllers responsive to endogenous proteins. Science 330:1251–1255. doi:10.1126/science.1192128
33. Davidson EA, van Balcom T, Levy M, Ellington AD (2010) Emulsion based selection of T7 promoters of varying activity. Pac Symp Biocomput, pp 433–443
34. De Mey M, Taymaz-Nikerel H, Baart G, Waegeman H, Maertens J, Heijnen JJ, van Gulik WM (2010) Catching prompt metabolite dynamics in *Escherichia coli* with the BioScope at oxygen rich conditions. Metab Eng 12:477–487
35. Dietrich JA, Yoshikuni Y, Fisher KJ, Woolard FX, Ockey D, McPhee DJ, Renninger NS, Chang MC, Baker D, Keasling JD (2009) A novel semi-biosynthetic route for artemisinin production using engineered substrate-promiscuous P450(BM3). ACS Chem Biol 4:261–267. doi:10.1021/cb900006h
36. Donohue TJ (2009) Targeted sigma factor turnover inserts negative control into a positive feedback loop. Mol Microbiol 73:747–750. doi:10.1111/j.1365-2958.2009.06823.x
37. Durand S, Storz G (2010) Reprogramming of anaerobic metabolism by the FnrS small RNA. Mol Microbiol 75:1215–1231
38. Engl C, Beek AT, Bekker M, de Mattos JT, Jovanovic G, Buck M (2011) Dissipation of proton motive force is not sufficient to induce the phage shock protein response in *Escherichia coli*. Curr Microbiol 62:1374–1385. doi:10.1007/s00284-011-9869-5
39. Feng X, Ding Y, Xian M, Xu X, Zhang R, Zhao G (2014) Production of optically pure d-lactate from glycerol by engineered *Klebsiella pneumoniae* strain. Bioresour Technol 172C:269–275. doi:10.1016/j.biortech.2014.09.074
40. Feng XJ, Hooshangi S, Chen D, Li G, Weiss R, Rabitz H (2004) Optimizing genetic circuits by global sensitivity analysis. Biophys J 87:2195–2202. doi:10.1529/biophysj.104.044131
41. Frey PA (1982) Mechanism of coupled electron and group transfer in *Escherichia coli* pyruvate dehydrogenase. Ann N Y Acad Sci 378:250–264
42. Fujisaki S, Takahashi I, Hara H, Horiuchi K, Nishino T, Nishimura Y (2005) Disruption of the structural gene for farnesyl diphosphate synthase in *Escherichia coli*. J Biochem 137:395–400. doi:10.1093/jb/mvi049
43. Garcia-Ochoa F, Castro EG, Santos VE (2000) Oxygen transfer and uptake rates during xanthan gum production. Enzyme and microbial technology 27:680–690
44. Gennis RB, Stewart V (1996) Respiration. In: Neidhardt FC, Curtiss R, Ingraham JL, Lin ECC, Low KB, Magasanik B, Reznikoff WS, Riley M, Schaechter M, Umbarger HE (eds) *Escherichia coli* and *salmonella*: cellular and molecular biology. ASM Press, Washington, DC, pp 217–261
45. Georgellis D, Kwon O, Lin EC (1999) Amplification of signaling activity of the arc two-component system of *Escherichia coli* by anaerobic metabolites. An in vitro study with different protein modules. J Biol Chem 274:35950–35954
46. Georgellis D, Kwon O, Lin EC (2001) Quinones as the redox signal for the arc two-component system of bacteria. Science 292:2314–2316
47. Georgellis D, Lynch AS, Lin EC (1997) In vitro phosphorylation study of the arc two-component signal transduction system of *Escherichia coli*. J Bacteriol 179:5429–5435
48. Gottesman S, McCullen CA, Guillier M, Vanderpool CK, Majdalani N, Benhammou J, Thompson KM, FitzGerald PC, Sowa NA, FitzGerald DJ (2006) Small RNA regulators and the bacterial response to stress. Cold Spring Harb Symp Quant Biol 71:1–11
49. Gottesman S, Storz G (2011) Bacterial small RNA regulators: versatile roles and rapidly evolving variations. Cold Spring Harb Perspect Biol 3(12):a003798. doi:10.1101/cshperspect.a003798
50. Guest JR, Angier SJ, Russell GC (1989) Structure, expression, and protein engineering of the pyruvate dehydrogenase complex of Escherichia coli. Ann N Y Acad Sci 573:76–99
51. Guest JR, Green J, Irvine AS, Sprio S (1996) The FNR modulon and FNR-regulated gene expression. Regulation of gene expression in *E coli*, pp 317–342
52. Gulmezian M, Hyman KR, Marbois BN, Clarke CF, Javor GT (2007) The role of UbiX in *Escherichia coli* coenzyme Q biosynthesis. Arch Biochem Biophys 467:144–153. doi:10.1016/j.abb.2007.08.009
53. Gunsalus RP (1992) Control of electron flow in *Escherichia coli*: coordinated transcription of respiratory pathway genes. J Bacteriol 174:7069–7074
54. Gunsalus RP, Park SJ (1994) Aerobic–anaerobic gene regulation in *Escherichia coli*: control by the ArcAB and Fnr regulons. Res Microbiol 145:437–450
55. Hakoshima T, Ichihara H (2007) Structure of SixA, a histidine protein phosphatase of the ArcB histidine-containing phosphotransfer domain in *Escherichia coli*. Methods Enzymol 422:288–304
56. Hasegawa S, Suda M, Uematsu K, Natsuma Y, Hiraga K, Jojima T, Inui M, Yukawa H (2013) Engineering of *Corynebacterium glutamicum* for high-yield L-valine production under oxygen deprivation conditions. Appl Environ Microbiol 79:1250–1257. doi:10.1128/AEM.02806-12
57. Hasegawa S, Uematsu K, Natsuma Y, Suda M, Hiraga K, Jojima T, Inui M, Yukawa H (2012) Improvement of the redox balance increases L-valine production by *Corynebacterium glutamicum* under oxygen deprivation conditions. Appl Environ Microbiol 78:865–875. doi:10.1128/AEM.07056-11
58. Haseltine EL, Arnold FH (2007) Synthetic gene circuits: design with directed evolution. Annu Rev Biophys Biomol Struct 36:1–19. doi:10.1146/annurev.biophys.36.040306.132600
59. Hasty J, McMillen D, Collins JJ (2002) Engineered gene circuits. Nature 420:224–230. doi:10.1038/nature01257
60. Hayashi T, Furuta Y, Furukawa K (2011) Respiration-deficient mutants of *Zymomonas mobilis* show improved growth and ethanol fermentation under aerobic and high temperature

conditions. J Biosci Bioeng 111:414–419. doi:10.1016/j.jbiosc.2010.12.009
61. Hayashi T, Kato T, Furukawa K (2012) Respiratory chain analysis of *Zymomonas mobilis mutants* producing high levels of ethanol. Appl Environ Microbiol 78:5622–5629. doi:10.1128/AEM.00733-12
62. Herzog B, Streckfuss-Bomeke K, Braus GH (2011) A feedback circuit between transcriptional activation and self-destruction of Gcn4 separates its metabolic and morphogenic response in diploid yeasts. J Mol Biol 405:909–925. doi:10.1016/j.jmb.2010.11.033
63. Hillmann F, Linde J, Beckmann N, Cyrulies M, Strassburger M, Heinekamp T, Haas H, Guthke R, Kniemeyer O, Brakhage AA (2014) The novel globin protein fungoglobin is involved in low oxygen adaptation of *Aspergillus fumigatus*. Mol Microbiol 93:539–553. doi:10.1111/mmi.12679
64. Iuchi S (1993) Phosphorylation/dephosphorylation of the receiver module at the conserved aspartate residue controls transphosphorylation activity of histidine kinase in sensor protein ArcB of *Escherichia coli*. J Biol Chem 268:23972–23980
65. Iuchi S, Lin EC (1991) Adaptation of *Escherichia coli* to respiratory conditions: regulation of gene expression. Cell 66:5–7
66. Iuchi S, Lin EC (1992) Mutational analysis of signal transduction by ArcB, a membrane sensor protein responsible for anaerobic repression of operons involved in the central aerobic pathways in *Escherichia coli*. J Bacteriol 174:3972–3980
67. Iuchi S, Matsuda Z, Fujiwara T, Lin EC (1990) The *arcB* gene of *Escherichia coli* encodes a sensor-regulator protein for anaerobic repression of the arc modulon. Mol Microbiol 4:715–727
68. Jervis AJ, Crack JC, White G, Artymiuk PJ, Cheesman MR, Thomson AJ, Le Brun NE, Green J (2009) The O2 sensitivity of the transcription factor FNR is controlled by Ser24 modulating the kinetics of [^{4}Fe-^{4}S] to [^{2}Fe-^{2}S] conversion. Proc Natl Acad Sci USA 106:4659–4664
69. Jeya M, Moon HJ, Lee JL, Kim IW, Lee JK (2010) Current state of coenzyme Q(10) production and its applications. Appl Microbiol Biotechnol 85:1653–1663. doi:10.1007/s00253-009-2380-2
70. Johnson K, Kleerebezem R, van Loosdrecht MC (2009) Model-based data evaluation of polyhydroxybutyrate producing mixed microbial cultures in aerobic sequencing batch and fed-batch reactors. Biotechnol Bioeng 104:50–67. doi:10.1002/bit.22380
71. Jojima T, Noburyu R, Sasaki M, Tajima T, Suda M, Yukawa H, Inui M (2015) Metabolic engineering for improved production of ethanol by *Corynebacterium glutamicum*. Appl Microbiol Biotechnol 99:1165–1172. doi:10.1007/s00253-014-6223-4
72. Jordan PA, Thomson AJ, Ralph ET, Guest JR, Green J (1997) FNR is a direct oxygen sensor having a biphasic response curve. FEBS Lett 416:349–352
73. Kaakoush NO, Miller WG, De Reuse H, Mendz GL (2007) Oxygen requirement and tolerance of *Campylobacter jejuni*. Res Microbiol 158:644–650. doi:10.1016/j.resmic.2007.07.009
74. Kadir TAA, Mannan AA, Kierzek AM, McFadden J, Shimizu K (2010) Modeling and simulation of the main metabolism in *Escherichia coli* and its several single-gene knockout mutants with experimental verification. Microb Cell Fact. doi:10.1186/1475-2859-9-88
75. Kang Y, Weber KD, Qiu Y, Kiley PJ, Blattner FR (2005) Genome-wide expression analysis indicates that FNR of *Escherichia coli* K-12 regulates a large number of genes of unknown function. J Bacteriol 187:1135–1160. doi:10.1128/JB.187.3.1135-1160.2005
76. Kim Y, Ingram LO, Shanmugam KT (2008) Dihydrolipoamide dehydrogenase mutation alters the NADH sensitivity of pyruvate dehydrogenase complex of *Escherichia coli* K-12. J Bacteriol 190:3851–3858
77. Kirkpatrick C, Maurer LM, Oyelakin NE, Yoncheva YN, Maurer R, Slonczewski JL (2001) Acetate and formate stress: opposite responses in the proteome of *Escherichia coli*. J Bacteriol 183:6466–6477
78. Koch-Koerfges A, Pfelzer N, Platzen L, Oldiges M, Bott M (2013) Conversion of *Corynebacterium glutamicum* from an aerobic respiring to an aerobic fermenting bacterium by inactivation of the respiratory chain. Biochim Biophys Acta 1827:699–708. doi:10.1016/j.bbabio.2013.02.004
79. Kodama Y, Watanabe K (2003) Isolation and characterization of a sulfur-oxidizing chemolithotroph growing on crude oil under anaerobic conditions. Appl Environ Microbiol 69:107–112
80. Kong MK, Lee PC (2011) Metabolic engineering of menaquinone-8 pathway of *Escherichia coli* as a microbial platform for vitamin K production. Biotechnol Bioeng 108:1997–2002. doi:10.1002/bit.23142
81. Koo BM, Yoon MJ, Lee CR, Nam TW, Choe YJ, Jaffe H, Peterkofsky A, Seok YJ (2004) A novel fermentation/respiration switch protein regulated by enzyme IIAGlc in *Escherichia coli*. J Biol Chem 279:31613–31621
82. Kumar R, Shimizu K (2010) Metabolic regulation of *Escherichia coli* and its *gdhA*, *glnL*, *gltB*, *D* mutants under different carbon and nitrogen limitations in the continuous culture. Microb Cell Fact 9:8. doi:10.1186/1475-2859-9-8
83. Kumar R, Shimizu K (2011) Transcriptional regulation of main metabolic pathways of *cyoA*, *cydB*, *fnr*, and *fur* gene knockout *Escherichia coli* in C-limited and N-limited aerobic continuous cultures. Microb Cell Fact 10:3. doi:10.1186/1475-2859-10-3
84. Kwon O, Georgellis D, Lin EC (2000) Phosphorelay as the sole physiological route of signal transmission by the arc two-component system of *Escherichia coli*. J Bacteriol 182:3858–3862
85. Lehtio L, Goldman A (2004) The pyruvate formate lyase family: sequences, structures and activation. Protein Eng Des Sel 17:545–552
86. Levskaya A, Chevalier AA, Tabor JJ, Simpson ZB, Lavery LA, Levy M, Davidson EA, Scouras A, Ellington AD, Marcotte EM et al (2005) Synthetic biology: engineering *Escherichia coli* to see light. Nature 438:441–442. doi:10.1038/nature04405
87. Li Y, Li M, Zhang X, Yang P, Liang Q, Qi Q (2013) A novel whole-phase succinate fermentation strategy with high volumetric productivity in engineered *Escherichia coli*. Bioresour Technol 149:333–340. doi:10.1016/j.biortech.2013.09.077
88. Liang JC, Bloom RJ, Smolke CD (2011) Engineering biological systems with synthetic RNA molecules. Mol Cell 43:915–926. doi:10.1016/j.molcel.2011.08.023
89. Lim CJ, Lee SY, Teramoto J, Ishihama A, Yan J (2013) The nucleoid-associated protein Dan organizes chromosomal DNA through rigid nucleoprotein filament formation in *E. coli* during anoxia. Nucleic Acids Res 41:746–753. doi:10.1093/nar/gks1126
90. Lin EC, Iuchi S (1991) Regulation of gene expression in fermentative and respiratory systems in Escherichia coli and related bacteria. Annu Rev Genet 25:361–387
91. Liu X, Jensen PR, Workman M (2012) Bioconversion of crude glycerol feedstocks into ethanol by *Pachysolen tannophilus*. Bioresour Technol 104:579–586. doi:10.1016/j.biortech.2011.10.065
92. Liu XJ, Liu RS, Li HM, Tang YJ (2012) Lycopene production from synthetic medium by *Blakeslea trispora* NRRL 2895 (+) and 2896 (−) in a stirred-tank fermenter. Bioprocess Biosyst Eng 35:739–749. doi:10.1007/s00449-011-0654-4
93. Locke JC, Young JW, Fontes M, Hernandez Jimenez MJ, Elowitz MB (2011) Stochastic pulse regulation in bacterial stress response. Science 334:366–369. doi:10.1126/science.1208144
94. Loiseau L, Gerez C, Bekker M, Ollagnier-de Choudens S, Py B, Sanakis Y, Teixeira de Mattos J, Fontecave M, Barras F (2007)

ErpA, an iron sulfur (Fe S) protein of the A-type essential for respiratory metabolism in *Escherichia coli*. Proc Natl Acad Sci USA 104:13626–13631
95. Luirink J, Yu Z, Wagner S, de Gier JW (2012) Biogenesis of inner membrane proteins in *Escherichia coli*. Biochim Biophys Acta 1817:965–976. doi:10.1016/j.bbabio.2011.12.006
96. Lynch AS, Lin EC (1996) Transcriptional control mediated by the ArcA two-component response regulator protein of *Escherichia coli*: characterization of DNA binding at target promoters. J Bacteriol 178:6238–6249
97. Mandin P, Gottesman S (2010) Integrating anaerobic/aerobic sensing and the general stress response through the ArcZ small RNA. EMBO J 29:3094–3107
98. Marchisio MA (2012) In silico implementation of synthetic gene networks. Methods Mol Biol 813:3–21. doi:10.1007/978-1-61779-412-4_1
99. Martin VJ, Pitera DJ, Withers ST, Newman JD, Keasling JD (2003) Engineering a mevalonate pathway in *Escherichia coli* for production of terpenoids. Nat Biotechnol 21:796–802. doi:10.1038/nbt833
100. Matsubara M, Mizuno T (2000) The SixA phospho-histidine phosphatase modulates the ArcB phosphorelay signal transduction in *Escherichia coli*. FEBS Lett 470:118–124
101. Mazumdar S, Bang J, Oh MK (2014) L-lactate production from seaweed hydrolysate of Laminaria japonica using metabolically engineered *Escherichia coli*. Appl Biochem Biotechnol 172:1938–1952. doi:10.1007/s12010-013-0653-9
102. Mazumdar S, Lee J, Oh MK (2013) Microbial production of 2,3 butanediol from seaweed hydrolysate using metabolically engineered *Escherichia coli*. Bioresour Technol 136:329–336. doi:10.1016/j.biortech.2013.03.013
103. Meganathan R (2001) Biosynthesis of menaquinone (vitamin K2) and ubiquinone (coenzyme Q): a perspective on enzymatic mechanisms. Vitam Horm 61:173–218
104. Meganathan R (2001) Ubiquinone biosynthesis in microorganisms. FEMS Microbiol Lett 203:131–139
105. Meyer AJ, Ellington AD (2011) Molecular evolution picks up the PACE. Nat Biotechnol 29:502–503. doi:10.1038/nbt.1884
106. Michel A, Koch-Koerfges A, Krumbach K, Brocker M, Bott M (2015) Anaerobic growth of *Corynebacterium glutamicum* via mixed-acid fermentation. Appl Environ Microbiol 81:7496–7508. doi:10.1128/AEM.02413-15
107. Mileykovskaya EI, Dowhan W (1993) Alterations in the electron transfer chain in mutant strains of *Escherichia coli* lacking phosphatidylethanolamine. J Biol Chem 268:24824–24831
108. Mills DC, Gundogdu O, Elmi A, Bajaj-Elliott M, Taylor PW, Wren BW, Dorrell N (2012) Increase in *Campylobacter jejuni* invasion of intestinal epithelial cells under low-oxygen coculture conditions that reflect the in vivo environment. Infect Immun 80:1690–1698. doi:10.1128/IAI.06176-11
109. Minohara S, Sakamoto J, Sone N (2002) Improved H^+/O ratio and cell yield of *Escherichia coli* with genetically altered terminal quinol oxidases. J Biosci Bioeng 93:464–469
110. Mobius K, Arias-Cartin R, Breckau D, Hannig AL, Riedmann K, Biedendieck R, Schroder S, Becher D, Magalon A, Moser J et al (2010) Heme biosynthesis is coupled to electron transport chains for energy generation. Proc Natl Acad Sci USA 107:10436–10441. doi:10.1073/pnas.1000956107
111. Moon TS, Lou C, Tamsir A, Stanton BC, Voigt CA (2012) Genetic programs constructed from layered logic gates in single cells. Nature 491:249–253. doi:10.1038/nature11516
112. Morris RL, Schmidt TM (2013) Shallow breathing: bacterial life at low O(2). Nat Rev Microbiol 11:205–212. doi:10.1038/nrmicro2970
113. Naz N, Mills DC, Wren BW, Dorrell N (2013) Enteric bacterial invasion of intestinal epithelial cells in vitro is dramatically enhanced using a vertical diffusion chamber model. J Vis Exp. doi:10.3791/50741
114. Newman JD, Marshall J, Chang M, Nowroozi F, Paradise E, Pitera D, Newman KL, Keasling JD (2006) High-level production of amorpha-4,11-diene in a two-phase partitioning bioreactor of metabolically engineered *Escherichia coli*. Biotechnol Bioeng 95:684–691. doi:10.1002/bit.21017
115. Newton NA, Cox GB, Gibson F (1972) Function of ubiquinone in *Escherichia coli*: a mutant strain forming a low level of ubiquinone. J Bacteriol 109:69–73
116. Nikel PI, Pettinari MJ, Galvagno MA, Mendez BS (2006) Poly(3-hydroxybutyrate) synthesis by recombinant *Escherichia coli arcA* mutants in microaerobiosis. Appl Environ Microbiol 72:2614–2620. doi:10.1128/AEM.72.4.2614-2620.2006
117. Nikel PI, Ramirez MC, Pettinari MJ, Mendez BS, Galvagno MA (2010) Ethanol synthesis from glycerol by *Escherichia coli* redox mutants expressing *adhE* from *Leuconostoc mesenteroides*. J Appl Microbiol 109:492–504. doi:10.1111/j.1365-2672.2010.04668.x
118. Nikel PI, Zhu J, San KY, Mendez BS, Bennett GN (2009) Metabolic flux analysis of *Escherichia coli creB* and *arcA* mutants reveals shared control of carbon catabolism under microaerobic growth conditions. J Bacteriol 191:5538–5548
119. Ogino T, Matsubara M, Kato N, Nakamura Y, Mizuno T (1998) An *Escherichia coli* protein that exhibits phosphohistidine phosphatase activity towards the HPt domain of the ArcB sensor involved in the multistep His-Asp phosphorelay. Mol Microbiol 27:573–585
120. Okino S, Noburyu R, Suda M, Jojima T, Inui M, Yukawa H (2008) An efficient succinic acid production process in a metabolically engineered *Corynebacterium glutamicum* strain. Appl Microbiol Biotechnol 81:459–464. doi:10.1007/s00253-008-1668-y
121. Okino S, Suda M, Fujikura K, Inui M, Yukawa H (2008) Production of D-lactic acid by *Corynebacterium glutamicum* under oxygen deprivation. Appl Microbiol Biotechnol 78:449–454. doi:10.1007/s00253-007-1336-7
122. Park SJ, Gunsalus RP (1995) Oxygen, iron, carbon, and superoxide control of the fumarase *fumA* and *fumC* genes of *Escherichia coli*: role of the *arcA*, *fnr*, and *soxR* gene products. J Bacteriol 177:6255–6262
123. Pasotti L, Politi N, Zucca S, Cusella De Angelis MG, Magni P (2012) Bottom-up engineering of biological systems through standard bricks: a modularity study on basic parts and devices. PLoS One 7:e39407. doi:10.1371/journal.pone.0039407
124. Peercy BE, Cox SJ, Shalel-Levanon S, San KY, Bennett G (2006) A kinetic model of oxygen regulation of cytochrome production in *Escherichia coli*. J Theor Biol 242:547–563
125. Pettinari MJ, Nikel PI, Ruiz JA, Mendez BS (2008) *ArcA* redox mutants as a source of reduced bioproducts. J Mol Microbiol Biotechnol 15:41–47
126. Pfleger BF, Pitera DJ, Smolke CD, Keasling JD (2006) Combinatorial engineering of intergenic regions in operons tunes expression of multiple genes. Nat Biotechnol 24:1027–1032. doi:10.1038/nbt1226
127. Portnoy VA, Herrgard MJ, Palsson BO (2008) Aerobic fermentation of D-glucose by an evolved cytochrome oxidase-deficient *Escherichia coli* strain. Appl Environ Microbiol 74:7561–7569. doi:10.1128/AEM.00880-08
128. Portnoy VA, Scott DA, Lewis NE, Tarasova Y, Osterman AL, Palsson BO (2010) Deletion of genes encoding cytochrome oxidases and quinol monooxygenase blocks the aerobic–anaerobic shift in *Escherichia coli* K-12 MG1655. Appl Environ Microbiol 76:6529–6540. doi:10.1128/AEM.01178-10
129. Ramos I, Perez R, Reinoso M, Torio R, Fdz-Polanco M (2014) Microaerobic digestion of sewage sludge on an industrial-pilot

scale: the efficiency of biogas desulphurisation under different configurations and the impact of O2 on the microbial communities. Bioresour Technol 164:338–346. doi:10.1016/j.biortech.2014.04.109
130. Randall A, Guye P, Gupta S, Duportet X, Weiss R (2011) Design and connection of robust genetic circuits. Methods Enzymol 497:159–186. doi:10.1016/B978-0-12-385075-1.00007-X
131. Reddy MV, Mohan SV (2012) Influence of aerobic and anoxic microenvironments on polyhydroxyalkanoates (PHA) production from food waste and acidogenic effluents using aerobic consortia. Bioresour Technol 103:313–321. doi:10.1016/j.biortech.2011.09.040
132. Rey FE, Harwood CS (2010) FixK, a global regulator of microaerobic growth, controls photosynthesis in *Rhodopseudomonas palustris*. Mol Microbiol 75:1007–1020. doi:10.1111/j.1365-2958.2009.07037.x
133. Salmon K, Hung SP, Mekjian K, Baldi P, Hatfield GW, Gunsalus RP (2003) Global gene expression profiling in *Escherichia coli* K12. The effects of oxygen availability and FNR. J Biol Chem 278:29837–29855
134. Salmon KA, Hung SP, Steffen NR, Krupp R, Baldi P, Hatfield GW, Gunsalus RP (2005) Global gene expression profiling in *Escherichia coli* K12: effects of oxygen availability and ArcA. J Biol Chem 280:15084–15096
135. Sanguinetti G, Ruttor A, Opper M, Archambeau C (2009) Switching regulatory models of cellular stress response. Bioinformatics 25:1280–1286
136. Schmitz RA, Achebach S, Unden G (2004) Analysis of fumarate nitrate reductase regulator as an oxygen sensor in *Escherichia coli*. Methods Enzymol 381:628–644
137. Shalel-Levanon S, San KY, Bennett GN (2005) Effect of ArcA and FNR on the expression of genes related to the oxygen regulation and the glycolysis pathway in *Escherichia coli* under microaerobic growth conditions. Biotechnol Bioeng 92:147–159
138. Shalel-Levanon S, San KY, Bennett GN (2005) Effect of oxygen, and ArcA and FNR regulators on the expression of genes related to the electron transfer chain and the TCA cycle in *Escherichia coli*. Metab Eng 7:364–374
139. Shalel-Levanon SS, San KY, Bennett GN (2005) Effect of oxygen on the *Escherichia coli* ArcA and FNR regulation systems and metabolic responses. Biotechnol Bioeng 89:556–564
140. Shan Y, Lai Y, Yan A (2012) Metabolic reprogramming under microaerobic and anaerobic conditions in bacteria. Sub-cellular Biochem 64:159–179. doi:10.1007/978-94-007-5055-5_8
141. Sharma P, Stagge S, Bekker M, Bettenbrock K, Hellingwerf KJ (2013) Kinase activity of ArcB from *Escherichia coli* is subject to regulation by both ubiquinone and demethylmenaquinone. PLoS One 8:e75412. doi:10.1371/journal.pone.0075412
142. Sharma P, Teixeira de Mattos MJ, Hellingwerf KJ, Bekker M (2012) On the function of the various quinone species in *Escherichia coli*. FEBS J 279:3364–3373. doi:10.1111/j.1742-4658.2012.08608.x
143. Shestopalov AI, Bogachev AV, Murtazina RA, Viryasov MB, Skulachev VP (1997) Aeration-dependent changes in composition of the quinone pool in *Escherichia coli*. Evidence of post-transcriptional regulation of the quinone biosynthesis. FEBS Lett 404:272–274
144. Shimizu K (2004) Metabolic flux analysis based on 13C-labeling experiments and integration of the information with gene and protein expression patterns. Adv Biochem Eng Biotechnol 91:1–49
145. Smolke CD, Martin VJ, Keasling JD (2001) Controlling the metabolic flux through the carotenoid pathway using directed mRNA processing and stabilization. Metab Eng 3:313–321. doi:10.1006/mben.2001.0194
146. Soballe B, Poole RK (1999) Microbial ubiquinones: multiple roles in respiration, gene regulation and oxidative stress management. Microbiology 145(Pt 8):1817–1830
147. Soballe B, Poole RK (1998) Requirement for ubiquinone downstream of cytochrome(s) b in the oxygen-terminated respiratory chains of *Escherichia coli* K-12 revealed using a null mutant allele of *ubiCA*. Microbiology 144(Pt 2):361–373
148. Sprinzak D, Elowitz MB (2005) Reconstruction of genetic circuits. Nature 438:443–448. doi:10.1038/nature04335
149. Tabor JJ, Levskaya A, Voigt CA (2011) Multichromatic control of gene expression in *Escherichia coli*. J Mol Biol 405:315–324. doi:10.1016/j.jmb.2010.10.038
150. Tanouchi Y, Pai A, You L (2009) Decoding biological principles using gene circuits. Mol BioSyst 5:695–703. doi:10.1039/b901584c
151. Teramoto J, Yoshimura SH, Takeyasu K, Ishihama A (2010) A novel nucleoid protein of *Escherichia coli* induced under anaerobiotic growth conditions. Nucleic Acids Res 38:3605–3618. doi:10.1093/nar/gkq077
152. Tolla DA, Savageau MA (2011) Phenotypic repertoire of the FNR regulatory network in *Escherichia coli*. Mol Microbiol 79:149–165
153. Tolla DA, Savageau MA (2010) Regulation of aerobic-to-anaerobic transitions by the FNR cycle in *Escherichia coli*. J Mol Biol 397:893–905
154. Tseng CP, Albrecht J, Gunsalus RP (1996) Effect of microaerophilic cell growth conditions on expression of the aerobic (*cyoABCDE* and *cydAB*) and anaerobic (*narGHJI, frdABCD*, and *dmsABC*) respiratory pathway genes in *Escherichia coli*. J Bacteriol 178:1094–1098
155. Tsuge Y, Yamamoto S, Kato N, Suda M, Vertes AA, Yukawa H, Inui M (2015) Overexpression of the phosphofructokinase encoding gene is crucial for achieving high production of D-lactate in *Corynebacterium glutamicum* under oxygen deprivation. Appl Microbiol Biotechnol 99:4679–4689. doi:10.1007/s00253-015-6546-9
156. Tybrandt K, Larsson KC, Richter-Dahlfors A, Berggren M (2010) Ion bipolar junction transistors. Proc Natl Acad Sci USA 107:9929–9932. doi:10.1073/pnas.0913911107
157. Unden G, Achebach S, Holighaus G, Tran HG, Wackwitz B, Zeuner Y (2002) Control of FNR function of *Escherichia coli* by O_2 and reducing conditions. J Mol Microbiol Biotechnol 4:263–268
158. Unden G, Becker S, Bongaerts J, Holighaus G, Schirawski J, Six S (1995) O2-sensing and O_2-dependent gene regulation in facultatively anaerobic bacteria. Arch Microbiol 164:81–90
159. Unden G, Bongaerts J (1997) Alternative respiratory pathways of *Escherichia coli*: energetics and transcriptional regulation in response to electron acceptors. Biochim Biophys Acta 1320:217–234
160. Unden G, Schirawski J (1997) The oxygen-responsive transcriptional regulator FNR of *Escherichia coli*: the search for signals and reactions. Mol Microbiol 25:205–210
161. Vaziri S, Lupina G, Henkel C, Smith AD, Ostling M, Dabrowski J, Lippert G, Mehr W, Lemme MC (2013) A graphene-based hot electron transistor. Nano Lett 13:1435–1439. doi:10.1021/nl304305x
162. Wagner AF, Schultz S, Bomke J, Pils T, Lehmann WD, Knappe J (2001) YfiD of *Escherichia coli* and Y06I of bacteriophage T4 as autonomous glycyl radical cofactors reconstituting the catalytic center of oxygen-fragmented pyruvate formate-lyase. Biochem Biophys Res Commun 285:456–462
163. Wallace BJ, Young IG (1977) Role of quinones in electron transport to oxygen and nitrate in *Escherichia coli*. Studies with a ubiA- menA-double quinone mutant. Biochim Biophys Acta 461:84–100

164. Wang Q, Ou MS, Kim Y, Ingram LO, Shanmugam KT (2010) Metabolic flux control at the pyruvate node in an anaerobic *Escherichia coli* strain with an active pyruvate dehydrogenase. Appl Environ Microbiol 76:2107–2114
165. Whistance GR, Threlfall DR (1968) Effect of anaerobiosis on the concentrations of demethylmenaquinone, menaquinone and ubiquinone in *Escherichia freundii*, *Proteus mirabilis* and *Aeromonas punctata*. Biochem J 108:505–507
166. Wissenbach U, Ternes D, Unden G (1992) An *Escherichia coli* mutant containing only demethylmenaquinone, but no menaquinone: effects on fumarate, dimethylsulfoxide, trimethylamine N-oxide and nitrate respiration. Arch Microbiol 158:68–73
167. Wu H, Bennett GN, San KY (2015) Metabolic control of respiratory levels in coenzyme Q biosynthesis-deficient *Escherichia coli* strains leading to fine-tune aerobic lactate fermentation. Biotechnol Bioeng 112:1720–1726. doi:10.1002/bit.25585
168. Wu H, Tuli L, Bennett GN, San KY (2015) Metabolic transistor strategy for controlling electron transfer chain activity in *Escherichia coli*. Metab Eng 28:159–168. doi:10.1016/j.ymben.2015.01.002
169. Wyborn NR, Messenger SL, Henderson RA, Sawers G, Roberts RE, Attwood MM, Green J (2002) Expression of the *Escherichia coli yfiD* gene responds to intracellular pH and reduces the accumulation of acidic metabolic end products. Microbiology 148:1015–1026
170. Zeng H, Snavely I, Zamorano P, Javor GT (1998) Low ubiquinone content in *Escherichia coli* causes thiol hypersensitivity. J Bacteriol 180:3681–3685
171. Zhang H, Javor GT (2003) Regulation of the isofunctional genes *ubiD* and *ubiX* of the ubiquinone biosynthetic pathway of *Escherichia coli*. FEMS Microbiol Lett 223:67–72
172. Zhu J, Sanchez A, Bennett GN, San KY (2011) Manipulating respiratory levels in *Escherichia coli* for aerobic formation of reduced chemical products. Metab Eng 13:704–712. doi:10.1016/j.ymben.2011.09.006
173. Zhu J, Shalel-Levanon S, Bennett G, San KY (2006) Effect of the global redox sensing/regulation networks on *Escherichia coli* and metabolic flux distribution based on C-13 labeling experiments. Metab Eng 8:619–627
174. Zhu J, Shalel-Levanon S, Bennett G, San KY (2007) The YfiD protein contributes to the pyruvate formate-lyase flux in an *Escherichia coli arcA* mutant strain. Biotechnol Bioeng 97:138–143
175. Zhuang K, Vemuri GN, Mahadevan R (2011) Economics of membrane occupancy and respiro-fermentation. Mol Syst Biol 7:500. doi:10.1038/msb.2011.34
176. Zimmermann HF, Anderlei T, Buchs J, Binder M (2006) Oxygen limitation is a pitfall during screening for industrial strains. Appl Microbiol Biotechnol 72:1157–1160. doi:10.1007/s00253-006-0414-6