Metabolic Regulation and Coordination of the Metabolism in Bacteria in Response to a Variety of Growth Conditions

Kazuyuki Shimizu

Abstract Living organisms have sophisticated but well-organized regulation system. It is important to understand the metabolic regulation mechanisms in relation to growth environment for the efficient design of cell factories for biofuels and biochemicals production. Here, an overview is given for carbon catabolite regulation, nitrogen regulation, ion, sulfur, and phosphate regulations, stringent response under nutrient starvation as well as oxidative stress regulation, redox state regulation, acid-shock, heat- and cold-shock regulations, solvent stress regulation, osmoregulation, and biofilm formation, and quorum sensing focusing on *Escherichia coli* metabolism and others. The coordinated regulation mechanisms are of particular interest in getting insight into the principle which governs the cell metabolism. The metabolism is controlled by both enzyme-level regulation and transcriptional regulation via transcription factors such as cAMP–Crp, Cra, Csr, Fis, P_{II}(GlnB), NtrBC, CysB, PhoR/B, SoxR/S, Fur, MarR, ArcA/B, Fnr, NarX/L, RpoS, and (p)ppGpp for stringent response, where the timescales for enzyme-level and gene-level regulations are different. Moreover, multiple regulations are coordinated by the intracellular metabolites, where fructose 1,6-bisphosphate (FBP), phosphoenolpyruvate (PEP), and acetyl-CoA (AcCoA) play important roles for enzyme-level regulation as well as transcriptional control, while α-ketoacids such as α-ketoglutaric acid (αKG), pyruvate (PYR), and oxaloacetate (OAA) play important roles for the coordinated regulation between carbon source uptake rate and other nutrient uptake rate such as nitrogen or sulfur uptake rate by modulation of cAMP via Cya.

Keywords Acetate overflow metabolism · Acid shock · Catabolite regulation · Heat shock · Nitrogen regulation · Osmoregulation · Oxidative stress · Oxygen limitation · Phosphate regulation · Redox regulation · Stringent response · Sulfur regulation

K. Shimizu (✉)
Kyushu Institute of Technology, Iizuka, Fukuoka 820-8502, Japan
e-mail: shimi@bio.kyutech.ac.jp; shimi@ttck.keio.ac.jp

K. Shimizu
Institute of Advanced Biosciences, Keio University, Tsuruoka, Yamagata 997-0017, Japan

Abbreviations

Metabolites

CIT	Citrate
E4P	Erythrose-4-phosphate
FBP	Fructose-1,6-bisphosphate
F1P	Fructose 1-phosphate
F6P	Fructose-6-phosphate
G6P	Glucose-6-phosphate
GAP	Glyceraldehyde-3-phosphate
GOX	Glyoxylate
ICI	Isocitrate
KDPG	2-keto-3-deoxy-6-phosphogluconate
αKG	α-ketoglutarate
MAL	Malate
OAA	Oxaloacetate
PEP	Phosphoenolpyruvate
6PG	6-phosphogluconate
PYR	Pyruvate

Protein and enzymes

Ack	Acetate kinase
Acs	Acetyl-coenzyme A synthetase
Adk	Adenylate kinase
CS	Citrate synthase
Cya	Adenylate cyclase
EI	Enzyme I
EII	Enzyme II
Fdp	Fructose bisphosphatase
FDH	Formate dehydrogenase
Fhl	Formate hydrogen lyase
GAD	Glutamate decarboxylase
G6PDH	Glucose-6-phosphate dehydrogenase
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GOGAT	Glutamate synthase
GS	Glutamine synthetase
HPr	Histidine-phosphorylatable protein
Hyc	Hydrogenase
ICDH	Isocitrate dehydrogenase
Icl	Isocitrate lyase
KGDH	α-ketoglutaric acid dehydrogenase
LDH	Lactate dehydrogenase
Mez	Malic enzyme
MS	Malate synthase
NOX	NADH oxidase

Pck	Phosphoenolpyruvate carboxykinase
PDH	Pyruvate dehydrogenase
Pfk	Phosphofructokinase
PGDH	6-phosphogluconate dehydrogenase
Pgi	Phosphoglucose isomerase
Pox	Pyruvate oxidase
Ppc	Phosphoenolpyruvate carboxylase
Pps	Phosphoenolpyruvate synthase
Pta	Phosphotransacetylase
Pyk	Pyruvate kinase
SOD	Superoxide dismutase

Others

ED pathway	Entner–Doudoroff pathway
EMP pathway	Embden–Meyerhof–Parnas pathway
PMF	Proton motive force
PP pathway	Pentose phosphate pathway
PTS	Phosphotransferase system
ROS	Reactive oxygen species
TCA cycle	Tricarboxylic acid cycle

Contents

1 Introduction

The living organisms on earth survive by manipulating the cell system in response to the change in growth environment by sensing signals of both external and internal states of the cell. The complex signaling networks interconvert signals or stimuli for the cell to function properly. The transfer of information in signal transduction pathways and cascades is evolved to respond to the variety of growth environment. Metabolism is the core for energy generation (catabolism) and cell synthesis (anabolism). Metabolic network, defined as the set and topology of metabolic biochemical reactions within a cell, plays an essential role for the cell to survive, where it is under organized control. The set of enzymes changes dynamically in accordance with the change in growth environment and the cell's state. The enzymes that form the metabolic pathways are subject to multiple levels of regulation, and it is becoming more and more important to deeply understand the overall regulation mechanism. This may be made by integrating different levels of OMICS information with the help of systems biology approach. Although huge amount of information is embedded in genome, only a subset of the pathways among possible topological networks is active at certain point in time under certain growth condition. The keen interest is how it is managed by the cell with coordination of the metabolism in response to the change in growth condition.

Recent investigation on the metabolism is widespread ranging from bacteria to human, where much attention is focused on cancer cell metabolism [1–4]. The metabolic capabilities allow various organisms to grow in various limiting conditions and environmental niches in the ecological biosphere [5–7]. Many efforts have been focused on the emerging challenges in sustainable energy, and green society, as well as pharmaceuticals for human health by modifying the metabolic pathways [8–15].

Deep understanding on the metabolic regulation mechanism is essential for all these efforts for manipulating and redesigning the metabolism, and it is critical to understand the basic principles which govern the cell metabolism [16–20]. Such principles may be in common to various organisms, or some set of organisms, while some are the specific to the organism of concern.

Biochemical logic of metabolic pathways may be determined based on key biochemical constraints such as thermodynamic favorability, availability of enzymatic mechanisms, and physicochemical properties of pathway intermediates [16]. More specifically, there might be a connection between an organism's growth environment and thermodynamic and biochemical properties for the determination of pathways [17]. How do organisms select the pathways among available pathways? For example, there are several glycolysis pathways such as Embden–Meyerhof–Parnas (EMP) pathway and Entner–Doudoroff (ED) pathway, but how is the pathway selected among them? The glucose metabolism may reflect a trade-off between a pathway's energy (ATP) yield and the amount of enzymatic protein required to catalyze the pathway flux. From this point of view, some microorganisms such as *Zymomonas mobilis* and *Pseudomonas* sp. mainly utilize ED

pathway instead of most popular energy-intensive EMP pathway due to less requirement of enzymatic protein together with thermodynamic preference [17].

In fact, the decision may be made not only by the above consideration, but it is also made by transcriptional regulation together with global regulators or transcription factors. Moreover, some specific metabolites are also involved for the coordinated regulation of the metabolism. Here, an overview is given for the metabolic regulation of microbes with special interest on the coordination of regulation systems.

2 Transport of Nutrient and Waste

The gram-negative bacteria such as *Escherichia coli* have two concentric membranes called outer and inner (or cytosolic) membranes surrounding the cytoplasm with the space called periplasm between these two membranes (Fig. 1). These membranes constitute a hydrophobic barrier against polar compounds. The outer membrane contains channel proteins, called porin proteins, where porins are substrate-specific, ion-selective, or nonspecific channels that allow the influx of small hydrophilic nutrient molecules and the efflux of waste products, antibiotics, and inhibitors [21, 22]. Among porins, OmpC and OmpF are the most abundant porins under typical growth condition. Their relative abundance changes depending on the osmolarity, temperature, and growth phase. Glucose is transported through these porins by diffusion when glucose concentration is higher above 0.2 mM [23, 24], while under glucose limitation, another porin LamB is induced, where LamB has high affinity to glucose [24].

The porin genes are under control of two-component system such as EnvZ–OmpR system, where EnvZ is an inner membrane sensor kinase, and OmpR is the cytoplasmic response regulator. In response to the environmental signals such as osmolarity, pH, temperature, nutrients, and toxins, EnvZ phosphorylates OmpR

Fig. 1 Glucose transport via PTS and other transporters
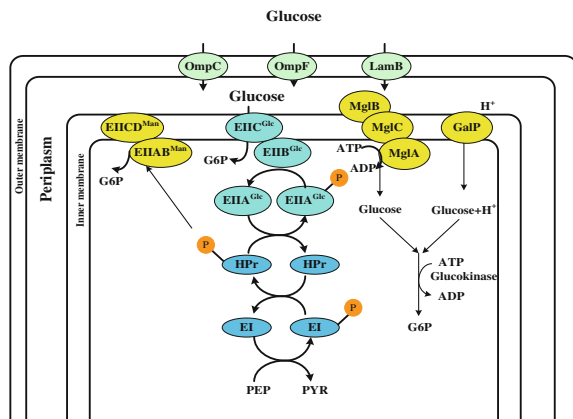
(OmpR-P), and OmpR-P activates the expression of such porin genes as *ompC* and *ompF* [21]. The outer membrane is important to protect the cell from harsh environmental condition.

The first step in the metabolism of carbohydrates is the transport of these molecules into the cytosol through inner or cytosolic membrane. Various carbohydrates can be transported by several mechanisms in bacteria [25]. The CO_2 and ammonia (NH_3)/ammonium (NH_4^+) may be transported through membrane by diffusion, where AmtB, which has the high affinity to ammonia, is induced and plays an important role for ammonia uptake under ammonia limitation. Without such a system, the important N-source, ammonia, is diffused out of the cell.

Primary transport of sugars is driven by ATP, while secondary transport is driven by electrochemical gradients of the translocated molecules across the membrane via symporters or anti-porters [26]. Sugar uptake by group translocation is unique for bacteria and is involved in the phosphotransferase system (PTS) as explained in the next section. So far, 21 PTSs have been identified in *E. coli*, while others are transported by non-PTS transporters [27]. The transporters for acids are rarely identified except formate (Foc) and acetate (ActP, YjcH), where other acids may be transported by diffusion. It is of interest to recognize [16] that charged compounds such as pyruvate, glycerate, and lactate may not be easily pass through hydrophobic lipid membrane [28–30]. On the other hand, uncharged molecules such as glucose, fructose, dihydroxyacetone, and glyceraldehydes can diffuse more freely through membrane [30–32]. Thus, a charged moiety such as phosphate group serves to reduce or prohibit their escape from the cell [33, 34]. From this point of view, it is of interest to see the fact that all the glycolysis, pentose phosphate (PP) pathway, and ED pathway metabolites are phosphorylated except pyruvate, while TCA cycle metabolites are not phosphorylated.

3 PTS and Carbon Catabolite Regulation

In the metabolic regulation, carbon catabolite regulation (CCR) plays far important roles from the points of view of energy acquisition and biomass synthesis, where PTS pays an important role [35]. In PTS, the phosphate is transferred from phosphoenolpyruvate (PEP) via successive phosphorelay reactions in turn by EI, HPr, EIIA^{Glc}, and EIICB^{Glc} to glucose (Fig. 1) [36]. Unphosphorylated EIIA^{Glc} inhibits the uptake of other carbohydrates by the so-called inducer exclusion [37, 38], while phosphorylated EIIA^{Glc} (EIIA^{Glc}-P) activates adenylate cyclase (Cya), which generates cyclic AMP (cAMP) from ATP, and leads to an increase in the intracellular cAMP level, where cAMP combines with the global regulator Crp (cAMP receptor protein) yielding cAMP–Crp complex, and plays an essential role for catabolite regulation. If the concentration ratio between PEP and pyruvate (PYR) (PEP/PYR) is high, EIIA^{Glc} is predominantly phosphorylated, while if this ratio is low, phosphorylated EIIA^{Glc} (EIIA^{Glc}-P) is dephosphorylated [39]. Catabolite repression

occurs not only by PTS-oriented regulation, but also by such catabolites as α-ketoacids as will be explained for the metabolic coordination.

Consider how the catabolite regulation affects the metabolism in response to the change in glucose consumption rate or the perturbation in the glycolysis activity. For this, it is useful to consider the continuous culture. In the continuous culture, as the dilution rate or the specific growth rate was increased, the glycolytic flux or the specific glucose consumption rate increases, and fructose 1,6-bisphosphate (FBP) concentration increases [40, 41]. The increased FBP allosterically enhances the activity of Pyk and Ppc by feed-forward control (Fig. 2a). Then, PEP concentration tends to be decreased due to the activation of Pyk and Ppc. PEP molecule allosterically inhibits Pfk activity by feedback regulation, and thus, the decrease in PEP concentration causes Pfk activity to be increased, and the glycolysis flux further increases, and in turn, FBP concentration increases more. Roughly speaking, in accordance with the increase in the glucose uptake rate, the intracellular metabolite pools of the upper glycolysis from glucose 6-phosphate (G6P) to glyceraldehydes 3-phosphate (GAP) increase, while those lower glycolysis from GAP to PEP decreases [40]. As stated above, there is a one-to-one relationship between the upper glycolysis flux and the FBP concentration. Moreover, the fluxes of lower glycolysis and the feed-forward activation of FBP on Pyk show the similar
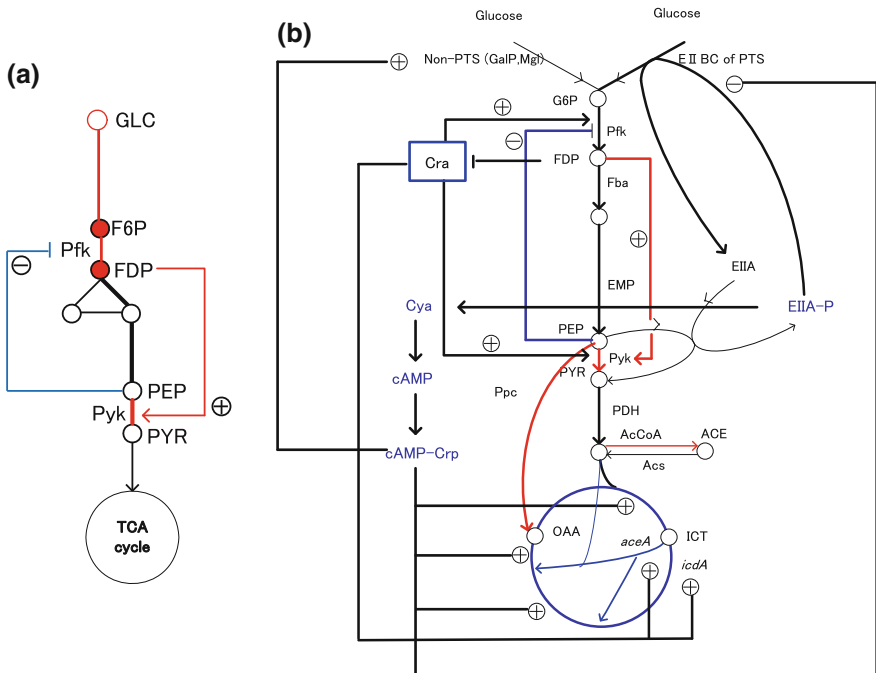

Fig. 2 Catabolite regulation of the central metabolism: **a** enzyme-level regulation, **b** overall enzyme-level and transcriptional regulations

relationship in the case of wild-type strain, where the metabolic fluxes may be considered to be sensed by molecular systems as "flux sensors" [41–43]. The meaning of this notion is that instead of preparing many nutrient-specific receptors for the cell to sense the variety of environmental signals, the flux-sensing system simply recognizes the fluxes by the specific intracellular metabolite as integral signal.

On the other hand, the decrease in PEP makes PEP/PYR ratio to be decreased. This causes EIIA^{Glc}-P to be dephosphorylated and in turn less activates Cya, and thus, cAMP is less formed. As a result, cAMP–Crp level decreases, which decreases the expression of *ptsG* (Table 1), and this causes the decrease in the glucose uptake rate. This forms a negative feedback loop for the initial increase in the glucose uptake rate [44, 45] (Fig. 2b). This indicates that PTS plays an essential role from the robustness point of view. Moreover, the decrease in cAMP–Crp also represses the expression of the TCA cycle genes (Table 1) (Fig. 2b).

In addition to cAMP–Crp, the catabolite repressor/activator protein (Cra) plays also an important role in the control of carbon flow in *E. coli* [46], where the carbon uptake and glycolysis genes are repressed, while gluconeogenic pathway genes are

Table 1 Effect of global regulators on the metabolic pathway gene expressions

Global regulator	Metabolic pathway genes
Cra	+: aceBAK, cydB, fbp, icdA, pckA, pgk, ppsA −: acnB, adhE, eda, edd, pfkA, pykF, zwf
Crp (cAMP–Crp)	+: aceEF, acnAB, acs, focA, fumA, gltA, lpdA, malT, manXYZ, mdh, mlc, pckA, pdhR, pflB, pgk, ptsG, sdhCDAB, sucABCD, ugpABCEQ, −: cyaA, lpdA, rpoS
ArcA/B	+: cydAB, focA, pflB −: aceBAK, aceEF, acnAB, cyoABCDE, fumAC, gltA, icdA, lpdA, mdh, nuoABCDEFGHIJKLMN, pdhR, sdhCDAB, sodA, sucABCD
Mlc	−: crr, manXYZ, malT, ptsG, ptsHI
PdhR	−: aceEF, lpdA
CsrA	+: eno, pfkA, pgi, pykF, tpiA −: fbp, glgC, glgA, glgB, pgm, ppsA, pckA,
Fur	−: entABCDEF, talB, sodA
RpoS	+: acnA, acs, ada, appAR, appB, argH, aroM, dps, bolA, fbaB, fumC, gabP, gadA, gadB, katE, katG, ldcC, narY, nuv, pfkB, osmE, osmY, poxB, sodC, talA, tktB, ugpE, C, xthA, yhgY, −: ompF
SoxR/S	+: acnA, cat, fumC, fur, sodA, sox, zwf
OxyR	+: ahpC, ahpF, katG:
PhoR/B	+: phoBR, phoA-psiF, asr, pstSCAB-phoU −: phoH, phnCHN, ugpA, argP
Fnr	+: acs, focA, frdABCD, pflB, yfiD −: acnA, cyoABCDE, cydAB, fumA, fnr, icdA, ndh, nuoABCEFGHIJKLMN, sdhCDAB, sucABCD

activated by Cra (Table 1). As mentioned above, the increase in the glucose uptake rate causes an increase in FBP concentration, where FBP inhibits Cra activity [47]. This causes the increase of the expression of the glycolysis genes such as *pfkA* and *pykF*, while it represses the expression of gluconeogenetic pathway genes such as *fbp*, *ppsA*, and *pckA* (Table 1), which implies acceleration of the glycolytic fluxes (Fig. 2b). The decrease in Cra activity also affects TCA cycle genes such that *icdA* and *aceA* gene expression is repressed, and thus, TCA cycle is further repressed by this mechanism (Fig. 2b). The increase in the glycolysis activity and the decrease in the TCA cycle activity cause more acetate production (Fig. 2b). This is the mechanism of acetate overflow metabolism in *E. coli*. The decrease in cAMP–Crp level also represses *acs* expression (Table 1), and this also causes acetate accumulation.

The question may arise as to why TCA cycle activity must be repressed in accordance with the increased activity of glycolysis. The cell growth rate might be more enhanced if the TCA cycle is not repressed, gaining more ATP from the increased NADH production at the TCA cycle. In fact, although the TCA cycle activity is repressed as mentioned above with the increase in glucose uptake rate [48–50], the absolute TCA cycle fluxes on the mmol basis tend to increase because the specific glucose consumption rate is increased [50]. This means that the respiration is activated with the increased production of NADH, and more reactive oxygen species (ROS) are generated in the respiratory chain and cause damage to the cell. This is the reason why the TCA cycle has to be repressed at higher glucose consumption rate. Of course, the cell furnishes a detoxifying system for ROS, where the transcription factors such as SoxR/S and OxyR play important roles for such oxidative stress regulation as will be mentioned later. The reducing equivalent, NADPH, plays an important role for detoxification of ROS as implied by the fact that SoxS activates *zwf* gene expression (Table 1). In the case of yeast and higher organisms, glutathione plays the similar role, where its production is enhanced by NADPH. The 6PGDH and G6PDH activities have been known to be the growth rate dependent [51], where the activation of the oxidative PP pathway is considered to be due to NADPH requirement for biosynthesis. However, the oxidative stress may also affect the activation of the oxidative PP pathway at higher cell growth rate. Moreover, some microbes such as *E. coli* produce NADPH at ICDH as well (instead of NADH) in the TCA cycle, where this is also related to the oxidative stress regulation as will be mentioned later.

4 Acetate Overflow Metabolism and the Reduction of Acetate Formation in *E. coli*

A major obstacle for the commercial scale production of useful recombinant proteins is the undesirable by-products formation. Among them, acetate formation is by far crucial in the case of *E. coli*, because it retards the cell growth, and it inhibits

protein formation. Moreover, such by-product formation causes a diversion of carbon that might otherwise have generated biomass or protein product [52].

The living organisms utilize energy in a highly efficient manner. However, some portion of energy is utilized for the cell maintenance, thus affecting the biomass yield. Recent studies on exact carbon balance together with thermodynamic arguments indicate that cells might have another avenue for energy utilization, where such phenomenon is called "energy spilling" [53, 54]. Under aerobic condition, *E. coli* mainly converts carbon source into biomass and CO_2, where the production of CO_2 is the loss of carbon, which determines the cell yield. A notable amount of carbon is also lost as acetate in particular at higher cell growth rate [49, 50, 55]. Another carbon wasting occurs toward pyrimidine pathway intermediates such as dihydroorotate, carbamoylaspartate, and orotate [54].

In *E. coli*, acetate is formed from acetyl-coenzyme A (AcCoA) by phospho-transacetylase (Pta) and acetate kinase (Ack) and from pyruvate by pyruvate oxidase (Pox) [56]. Acetate can be metabolized to AcCoA either by the reversed reactions of Pta–Ack or by acetyl-CoA synthetase (ACS) (Fig. 3a). Acetate formation has been known to be due to metabolic imbalance, also known as overflow metabolism as mentioned before, where the rate of acetyl-coenzyme A (AcCoA) formation via glycolysis surpasses the capacity of the TCA cycle in *E. coli* [57]. The mechanism of reducing the accumulated AcCoA, thus reducing acetate formation, is embedded in the cell metabolism. Namely, the anaplerotic pathway enzyme Ppc is allosterically activated by FBP and AcCoA [58], where the accumulated AcCoA activates Ppc, thus reducing PEP concentration, and in turn, the incoming flux through Pyk–PDH reduces, while the outgoing flux through citrate synthase (CS) increases caused by the increased OAA due to the activated Ppc
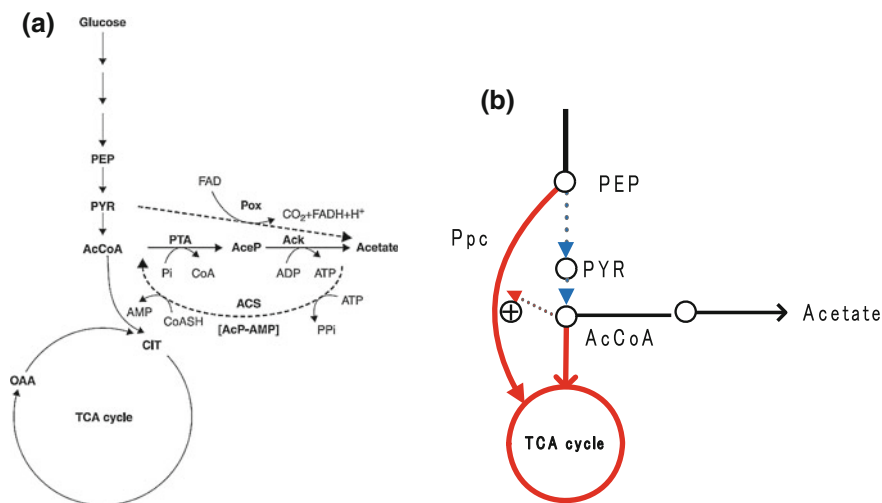

Fig. 3 Acetate metabolism: **a** acetate producing and consuming pathways, **b** homeostasis of AcCoA

(Fig. 3b). This means that AcCoA pool is in principle kept constant by such homeostatic regulation.

Since recombinant protein production is related to biomass formation, much attention has been paid on high cell density culture with reduced acetate formation [59]. The conventional approach to avoid or reduce acetate formation and attain high cell density culture is to reduce the glycolysis flux by keeping the substrate concentration at low level by fed-batch operation. The similar approach has been considered for baker's yeast cultivation to avoid ethanol production caused by Crabtree effect. The typical fed-batch culture is performed by employing feed-forward-type exponential and constant feeding profile [60], or feedback type of DO-stat [61, 62] and pH-stat [63]. In any case, feed-forward strategy must be compensated by feedback control. However, DO-stat and pH-stat strategies are based on the signals of substrate limitation, and the substrate-limited growth causes excessive energy consumption for the cell maintenance [64]. Moreover, in large-scale fed-batch culture, the substrate is distributed in space in the culture broth, and the gradients of substrate concentrations affect the cell growth, product formation, and cell viability [60, 65–67].

Another approach to reduce the glucose uptake rate is to modulate the substrate uptake pathway such as PTS, where the PTS mutation together with activation of other transporters can contribute for recombinant protein production with reduced acetate formation [52]. However, the cell growth rate inherently decreases in such mutants. Moreover, the regulation system as illustrated in Fig. 2b is not guaranteed for the robustness.

Since the main reason of acetate overflow metabolism is the repression of the TCA cycle caused by the increased substrate uptake rate as explained above, one idea is to activate TCA cycle by the knockout of *arcA* gene which codes for ArcA, and activate Acs by *pka* gene knockout [68]. However, the cell yield decreases and the cell growth rate decreases as will be also mentioned later for the roles of redox regulators such as ArcA/B.

5 Catabolite Regulation for the Uptake of Various Carbon Sources

The metabolite regulation differs depending on the carbon sources used, and this also affects acetate metabolism. Here, consider this for the typical carbon sources often used for biofuels and biochemicals production such as glycerol, xylose, fructose, and arabinose.

Glycerol, a rather "energy-poor" carbon source, has been paid recent attention for the production of biofuels and biochemicals such as 1,3-propanediol, 2,3-butanediol, ethanol, n-butanol, organic acids, and polyol, since it is a by-product of the biodiesel production [69–73]. In *E. coli*, glycerol is transported and phosphorylated to produce dihydroxy acetone phosphate (DHAP) of the central metabolism via the pathways

encoded by *glpF*, *glpK*, and *glpD*, where ATP (or in certain cases PEP) is used for the phosphorylation at GlpK reaction, while NADH is produced at GlpD reaction (Fig. 4a). These genes are under catabolite regulation by cAMP–Crp, so that glycerol is assimilated after glucose was depleted if glucose coexists. In the case of using glycerol as a single carbon source, cAMP–Crp increases by the mechanism as mentioned before due to the increase in the phosphorylated $EIIA^{Glc}$, where cAMP–Crp induces *glpF*, *glpK*, and *glpD* genes via *glpR*. Proteomic and enzymatic assay studies for the case of using rich media, fructose bisphosphatase (Fdp), the gluco-neogenesis enzyme in the upper glycolysis is activated for the fluxes toward PP pathway, while glyoxylate shunt is repressed [74]. Since FBP concentration decreases in the case of using glycerol as a carbon source, Cra is activated, and this together with up-regulation of cAMP–Crp causes *pckA* gene expression as well as TCA cycle gene expression to be up-regulated [75]. Moreover, *pykA* gene expression is activated instead of *pykF* gene in the case of using glycerol [75], where *pykF* is repressed by Cra, while *pykA* is activated by AMP.

The metabolism slightly changes depending on the strain. For example, in *E. coli* JM101 cultivated in minimal medium using glycerol as a carbon source, co-consumption of acetate and glycerol occurs, where the acetate produced via Pox is utilized via Acs and glyoxylate shunt, and little acetate is produced [73]. This may be caused by the higher levels of cAMP–Crp and Cra.

In the case of using glycerol as a carbon source, the glycerol uptake rate is low, and thus, the cell growth rate is also low. This is the main drawback of using glycerol as a carbon source in practice. The slow uptake rate of glycerol is due to allosteric inhibition of GlpK by FBP, where this may be considered to avoid the toxic methylglyoxal production caused by the accumulation of DHAP (Fig. 4a) [76]. The glycerol uptake rate can be increased by modulating GlpK by evolutional mutation with relaxing of feedback inhibition of GlpK by FBP [76]. However, as the glycerol uptake rate is increased, and the cell growth rate is increased, the phosphate of PEP or EI-P may be used for the phosphorylation at GlpK reaction, and thus, the phosphorylation level of $EIIA^{Glc}$ decreases, and in turn, cAMP level decreases and represses TCA cycle, causing acetate overflow metabolism [77].

In the case of using fructose, it is transported by fructose PTS, which has its own HPr-like protein domain called FPr. Namely, the phosphate of PEP is first transferred to EI (as EI-P), but then, this phosphate is transferred to FPr instead of HPr, and in turn, the phosphate is transferred via fructose-specific $EIIA^{Frc}$ and $EIIBC^{Frc}$ to fructose, where phosphorylated fructose becomes fructose 1-phosphate (F1P), where F1P inhibits Cra activity [47]. The *fruBKA* operon is under the control of cAMP–Crp, and thus, glucose is preferentially consumed by glucose PTS when glucose coexists, while this operon is repressed by Cra (Fig. 4b) [78]. Because of this, *Cra* gene knockout enables co-consumption of glucose and fructose with fructose to be consumed faster as compared to glucose (Fig. 5) [79]. Why was the fructose consumed faster than glucose, although the glycolysis activity increases upon *Cra* gene knockout [80, 81]? The possible scenario might be as follows: Namely, the activated FPr in *Cra* mutant competes with HPr (for glucose phosphorylation) for the phosphate of EI-P. Since phosphorylation of $EIIA^{Glc}$ via HPr
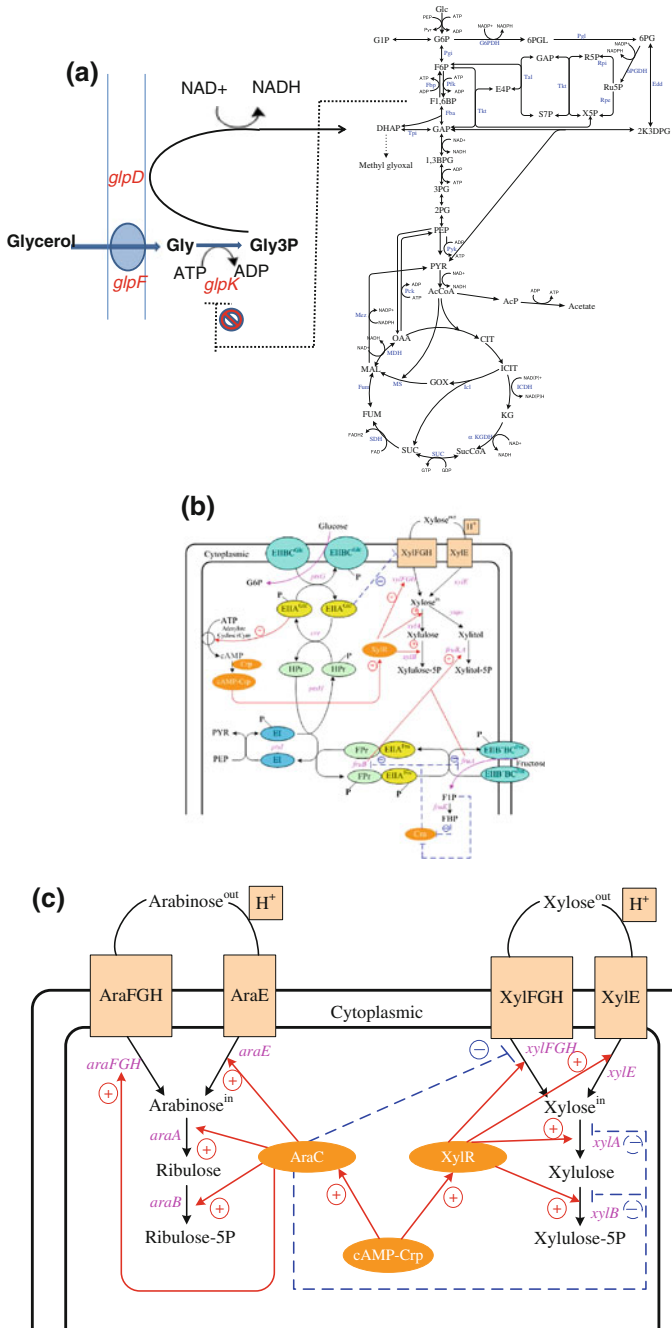
Fig. 4 Carbon sources assimilation and the hierarchical regulation for multiple carbon sources: **a** glycerol assimilating pathways, **b** assimilation of glucose, fructose, and xylose and their regulations [304], **c** assimilation of arabinose and xylose and their regulations [304]
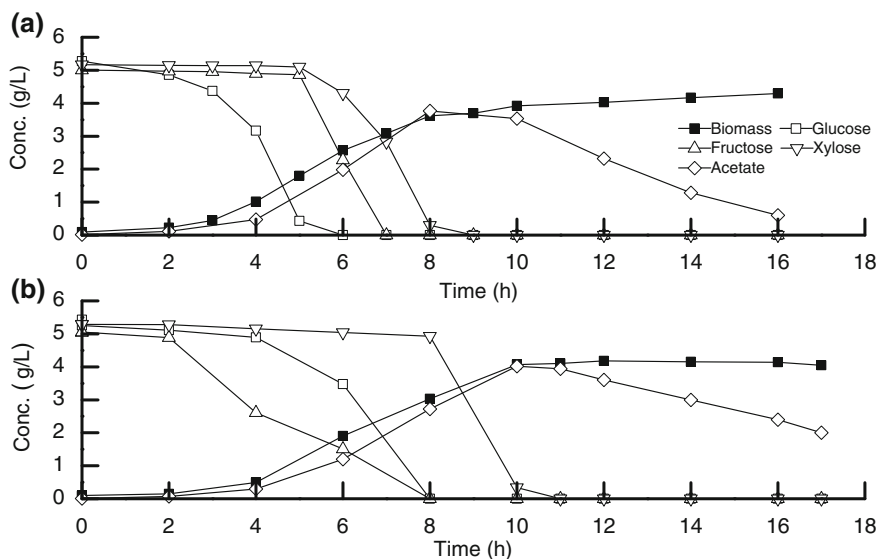
Fig. 5 Batch aerobic culture of wilt-type *E. coli* **a** and its *cra* mutant **b** for multiple carbon sources of glucose, fructose, and xylose [79]

becomes lower [82], the glucose uptake rate decreases as compared to the wild-type strain [79]. However, it is not still clear about the detailed mechanism on why catabolite repression is relaxed by *Cra* gene knockout.

In the case of using xylose as a carbon source, it is transported either by an ATP-dependent high-affinity ABC transporter encoded by *xylFGH* or by ATP-independent low-affinity proton symporter encoded by *xylE* (Fig. 4b) [83, 84]. In the case of xylose utilization, the transcription factor XylR regulates *xylAB/xylFGH* [85], where *xylR* is under the control of cAMP–Crp, and thus, catabolite repression occurs when glucose coexists, where glucose is preferentially consumed first. In the case when fructose coexists, phosphorylation of fructose is made via FPr, reducing the phosphorylation of HPr, and cAMP–Crp level becomes lower due to less phosphorylation of EIIA^{Glc}, and in turn, fructose is consumed faster as compared to xylose consumption (Fig. 5) [79]. In the case of anaerobic fermentation, NADH reoxidation and substrate-level phosphorylation for ATP generation are important, and ATP generation by Ack pathway is critical for survival in the case of using only xylose as a carbon source [86].

As for the assimilation of arabinose, the transporters are encoded by *araE*, *araFGH*, and *araJ* [87–89], while *araBAD* encodes arabinose catabolic enzymes. The arabinose system is under control of cAMP–Crp and AraC, where *araC* gene expression is activated by L-arabinose [90, 91].

As mentioned previously, the selective carbon source assimilation among available carbon sources forms a hierarchy, where glucose utilization is the highest priority in *E. coli*. Among pentose sugars such as xylose and arabinose, arabinose is

the preferred carbon source as compared to xylose [92, 93], where the transcription factor AraC represses *xylR*, while *araC* is under control of cAMP–Crp (Fig. 4c) [93]. This hierarchy changes by the specific gene knockout such as *Cra* gene knockout as mentioned above.

6 Transition of the Metabolism During Batch Culture

Since most of the industrial fermentations are conducted in batch mode, it is important to understand how the metabolism changes with respect to time, where various nutrient limitation and environmental stresses change and affect the metabolism [18, 19]. The ^{13}C-metabolic flux analysis is useful to track the change in the metabolic flux distribution during batch cultivation [94]. The typical growth condition changes from glucose-rich to acetate-rich condition and changes further to carbon-starved condition in the batch culture (Fig. 6). This requires a significant reorganization of the central metabolism. Since it invokes network-wide metabolic adaptation, it is one of the current targets of systems biology [42, 95].

Although the molecular mechanism underlying the metabolic transition from glucose to acetate has been extensively investigated in *E. coli* [56], its dynamics with respect to the sequence and timing of the molecular events have been poorly understood. Recently, this has been clarified to some extent, where the timescales for gene expression and enzyme regulation with metabolites are different [96]. Since it is critical for the cell to efficiently and quickly reprogram the metabolism for efficiently assimilating the nutrients under ever-changing environmental condition, the cell must have the elaborate managing system to cope with the environmental changes. In particular, enzyme-level regulation plays an important role for this, where Pyk and Ppc are allosterically activated by FBP as mentioned before. After glucose depletion, FBP concentration decreases accordingly, and Ppc and Pyk activities decrease by allosteric regulation, and PEP consumption is almost completely turned off. These make PEP concentration to be increased, and this buildup of PEP is kept nearly constant during certain period, and this may serve to quickly uptake the glucose by PTS if it becomes available again (Fig. 6b) [94, 97]. This mechanism is important for the fed-batch culture compensated by DO-stat or pH-stat, where carbon limitation often occurs periodically, and the uptake of carbon source can be made quickly and efficiently without delay giving little damage to the cell, while this may not be the case for the cells without having such regulation mechanism.

During batch culture, several transcription factors such as cAMP–Crp, Cra, Fis, Csr, RpoS, RpoD together with RelA and SpoT play important roles for catabolite and nutrient regulation in coordination among them depending on the growth phases. Considering the roles of such transcription factors, where cAMP–Crp and Cra have already been explained, carbon storage regulator (Csr) is explained in the next section.
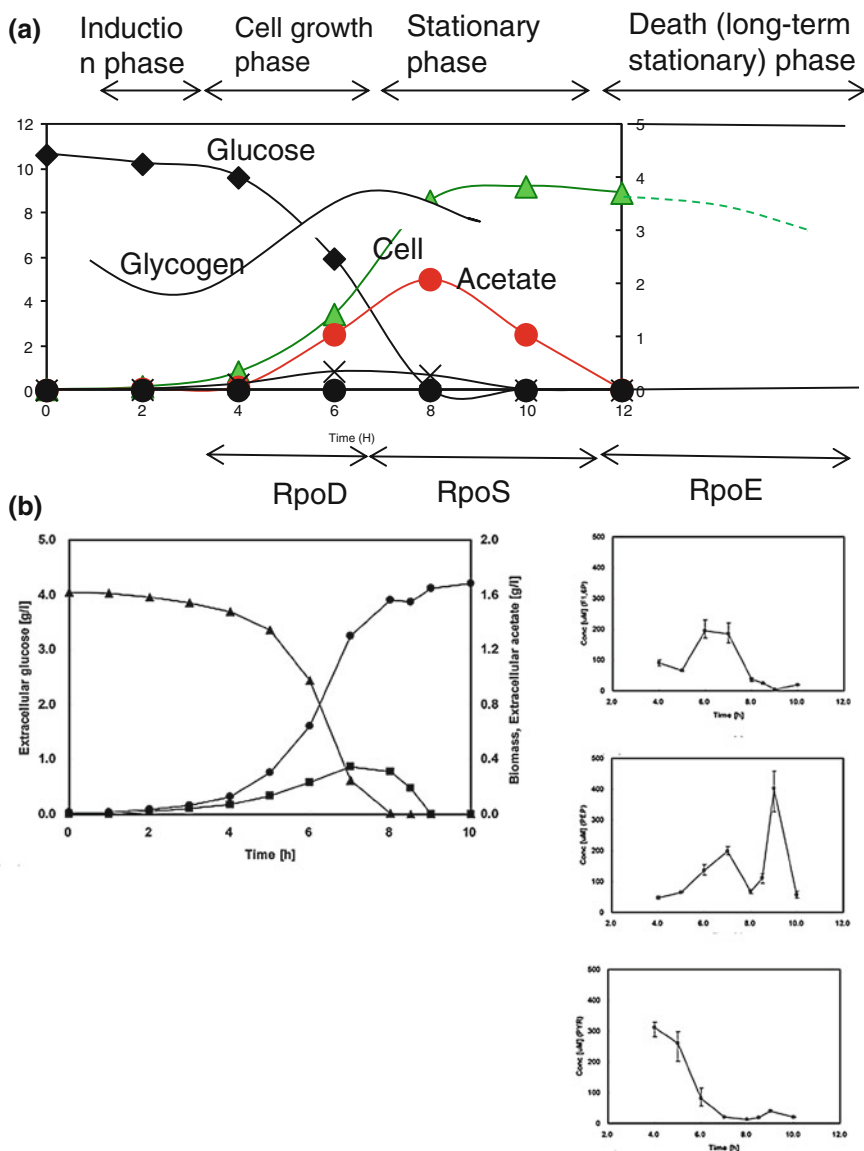
Fig. 6 Typical time profile for the batch culture of *E. coli*: **a** change in growth phase and σ factors, **b** change in intracellular metabolite concentrations for FDP, PEP, and PYR [94]

In *E. coli*, Fis (factor for inversion stimulation) is the most abundant during exponential growth phase [98]. Fis levels peak during early growth phase and thereafter decrease until they become very low during stationary phase [99], where Fis transcription is repressed by the stringent response [100], and Fis is subject to

growth rate control [101]. The stringent control and the growth control all require the stringent response regulator DksA [101], where (p)ppGpp (guanosine 3′,5′-bisphosphate including penta-phosphate pppGpp) and DksA interact with RNAP [102]. Fis plays a widespread role in signaling conditions of high nutritional control and outfitting the cells for efficient nutrient uptake and rapid growth [99]. Fis also plays a role in signaling poor nutritional condition, where in response to amino acid starvation, Fis is subject to severe and rapid negative control by the stringent response [101].

Bacteria generally have distinct strategies for the starvation in different nutrient sources. The individual hunger responses may be superimposed on a common protective starvation response [103]. Carbon limitation occurs at the onset of the stationary phase and leads to amino acid limitation, which requires the signaling pathways via RelA and SpoT during carbon and amino acid limitation [60]. During stringent response, nutrient limitation leads to the accumulation of ppGpp [104], which may bind to RNA polymerase [105], where ribosomal RNA and proteins are negatively regulated by ppGpp, which implies that protein biosynthesis declines, and in turn, the cell growth rate decreases. During amino acid limitation, (p)ppGpp is mediated by RelA. The accumulation of (p)ppGpp depends on the dual activity of SpoT as (p)ppGpp-hydrolase or ppGpp synthetase (Fig. 7). SpoT is activated in response to fatty acid starvation, carbon source starvation, diauxic shifts, phosphate limitation, ion limitation, hyper-osmotic shock, and oxidative stress [106].

The alamone ppGpp is involved in the regulation of σ^S on the transcriptional and posttranscriptional level [107], where ppGpp concentration increases with lower growth rates and affects RpoS, and ppGpp accumulates immediately after onset of nutrient starvation. The elevation of σ^S negatively regulates σ^D-dependent house-keeping genes [108]. Moreover, ppGpp influences the competition between different stress-related sigma factors in the binding of RNA polymerase core enzyme at the expense of σ^D [109] and RNA polymerase availability [60]. RpoS plays an important role at the stationary phase or carbon-starved conditions as well as other
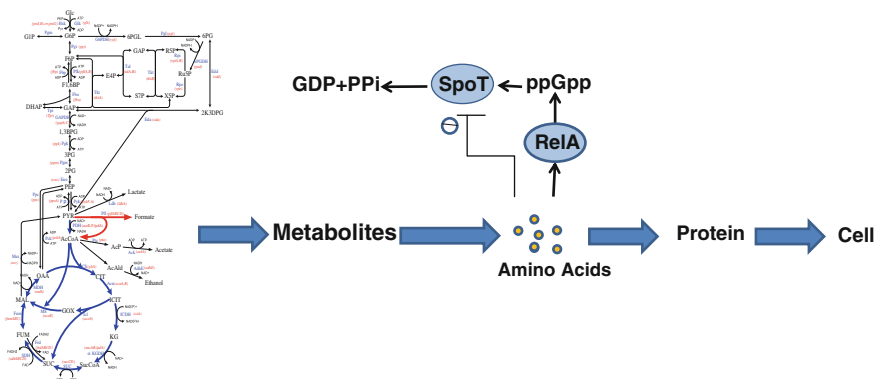

Fig. 7 Stringent response under amino acid limitation and regulation

stress conditions in *E. coli* [107, 110, 111]. Under normal situation with rich media, RpoS is rapidly degraded by ClpXP proteases, and the proteolytic activity of this enzyme is considerably reduced [107, 110, 111]. RpoS tends to increase as the cultivation proceeds from late growth phase to the stationary phase of the batch culture [112, 113].

After the stationary phase in the batch culture, the death phase and long-term stationary phase follow [114]. During the stationary phase, nutrient becomes exhausted, and waste products gradually accumulate, which may become a stress to the cell, and this eventually leads to the death phase in which the number of viable and culturable cells declines. Since majority of the cells in the death phase are viable but nonculturable or dying, nutrients from a portion of such cells are released into the medium. The released nutrients support the survival of the remaining culturable cells, and viable and culturable cells can survive for months or years in the long-term stationary phase [115, 116]. The σ^E-dependent cell lysis is to eliminate damaged cells in the stationary phase in *E. coli* [116], where the cell lysis proceeds in the cascade of $\sigma^E \rightarrow$ expression of *micA* and *rybB* $\rightarrow$ reduction in Omp proteins in the outer membrane $\rightarrow$ disintegration of outer membrane [117]. The cell lysis cascade appears to be related to the oxidative stress in the early stationary phase [118].

7 Carbon Storage Regulation

In the batch culture, the glycogen decreases during early growth phase or induction phase and increases at the late growth phase (Fig. 6) [119]. In the typical batch culture, glucose consumption rate is low during early growth phase or the induction phase, which may be due to glycogen utilization during unbalanced nutrient condition. Moreover, glycogen is accumulated at the late growth phase when the carbon source is going to be limited, which may be due to the preparation of carbon source as glycogen to be used under carbon source starvation. Csr plays an important role for such phenomenon.

The Csr system influences a variety of physiological processes such as central carbon metabolism, biofilm formation, motility, peptide uptake, virulence and pathogenesis, quorum sensing, and oxidative stress response [120–123]. Csr is controlled by the RNA binding protein CsrA, a posttranscriptional global regulator that regulates mRNA stability and translation [123], where CsrA is regulated by two sRNAs such as CsrB and CsrC [124–126]. CsrA regulates the central carbon metabolism and glycogenesis such that glycogen synthesis pathway gene expression, as well as gluconeogenic pathway gene expression, is repressed, while glycolysis gene expression is activated [120, 127] (Table 1).

Two sigma factors such as σ^{70} and σ^S affect *csrA* gene expression [122, 128]. In fact, the strong positive effects of ppGpp and DksA on *csrB/C* transcription and negative effects of CsrA on RelA expression and (p)ppGpp accumulation during stringent response [129]. This suggests that CsrB through CsrA directly regulates

DksA, thereby forming a positive feedback loop, and also, DksA and ppGpp activate the expression of *csr* genes [129], indicating the links between CsrA/B and the stringent response [129, 130].

The *csr* gene knockout affects the central metabolism such that the glycolysis activity is repressed (and the oxidative PP pathway is activated) together with acetate formation in the case of using glucose as a carbon source [131]. In the case of using gluconate as a carbon source, ED pathway is exclusively used for *csrA* mutant [131]. In the above cases, FBP concentration decreases [131], and thus, Cra may be activated and affects the metabolism by repressing glycolysis genes.

The *csrA* gene disruption also causes a significant increase in PEP concentration, since CsrA activates *pykF* gene expression, while it represses *pckA* and *ppsA* genes. The precursors of shikimate pathway for aromatic amino acids formation are a single E4P and two PEP molecules, and thus, over-expression of *tktA* with *csrA* gene disruption enhances phenylalanine biosynthesis [132].

Moreover, biofuels production can be improved by the over-expression of CsrB by activating native fatty acid and heterologous n-butanol and isoprenoid pathways [130]. In particular, CsrB-mediated degradation of CsrA drives over-expression of *glgCAP* operon, which results in the accretion of the storage polysaccharide glycogen.

8 Nitrogen Regulation

Next to carbon (C) source metabolism, nitrogen (N) metabolism is important to understand the cell metabolism. The N-regulation is controlled by σ^{54} encoded by *rpoN*. The main players in the hierarchical network for nitrogen metabolism and regulation are the ammonia transporter AmtB and a glutamine transporter GlnHPQ, metabolic pathways such as glutamate dehydrogenase (GDH) encoded by *gdhA*, glutamine synthetase (GS) encoded by *glnA*, and glutamate synthase (GOGAT) encoded by *gltBD*, the two bifunctional enzymes such as adenylyl transferase/ adenylyl-removing enzyme (ATase) and uridylyl transferase/uridylyl removing enzyme (UTase), the two-component regulatory system composed of the histidine protein kinase, nitrogen regulator II (NR_{II}) encoded by *glnL* and the response regulator I (NR_I) encoded by *glnG*, three global transcriptional regulators such as nitrogen assimilation control (Nac) protein, leucine-responsive regulatory protein (Lrp), and Crp, the glutaminases, and the nitrogen phosphotransferase system [133].

N-source such as ammonia (NH_3)/ammonium (NH_4^+) is predominantly assimilated at glutamate dehydrogenase (GDH) reaction, where α-ketoglutarate (αKG) is converted to glutamic acid (Glu), where NADPH is required for this reaction (Fig. 8). Then, glutamate is converted to glutamine (Gln) at glutamate synthetase (GS) reaction, where NH_3/NH_4^+ and ATP are required for this reaction. Thus, the flux goes from αKG via Glu to Gln, and thus, Gln accumulates under excess ammonia condition. Under N-limitation, the expression of *gdhA*, which encodes GDH, is repressed by Nac, and thus, Gln concentration decreases, and αKG
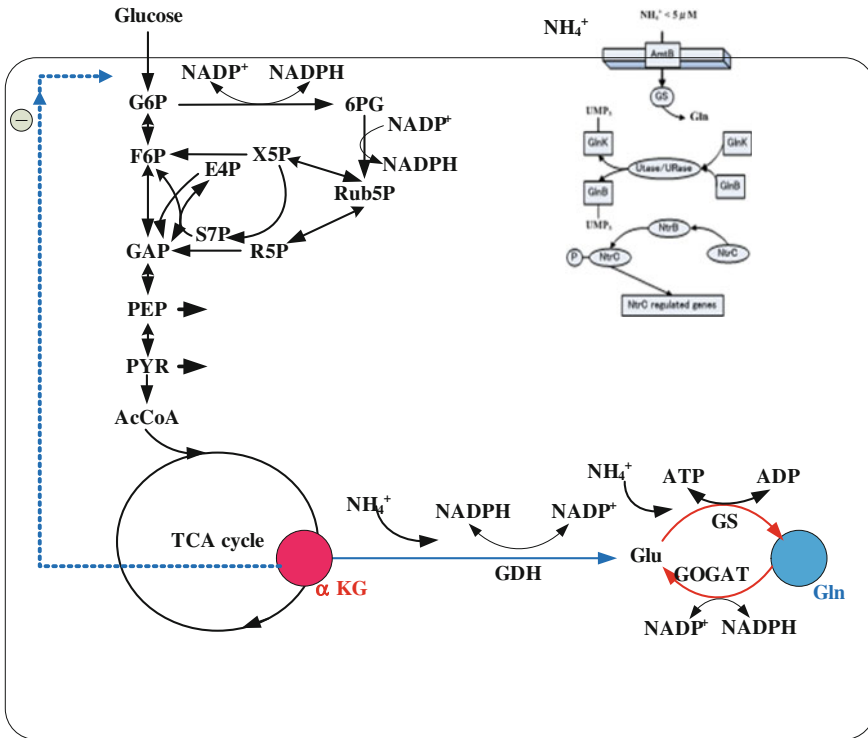
Fig. 8 Ammonia transport via AmtB, ammonia assimilation, and the effect of αKG on the glucose PTS under N-limitation

accumulates, where glutamate is formed from Gln by glutamate synthase (GOGAT) reaction (Fig. 8). Namely, under N-limitation, GS/GOGAT cycle plays an important role.

Intracellular ammonium is assimilated into biomass in two steps: Namely, it is first captured in the form of glutamic acid using carbon skeleton of αKG via GS/GOGAT cycle. Then, N-group in glutamate is transferred by aminotransferase reactions to synthesize other amino acids thus incorporating into biomass, while recycling the carbon skeleton back to αKG [134]. The αKG pool, which integrates imbalance between the ammonium assimilation flux and the biomass incorporation flux, activates AmtB [135–137] via GlnK. If ammonia level drops, then the rate of ammonia assimilation will drop immediately, which results in αKG accumulation [138]. When extracellular ammonia concentration is low around 5 μM or less, ammonia is captured and transported into the cell via AmtB and is converted to glutamine by GS, and UTase uridylylates both GlnK and GlnB [139] (Fig. 9). When extracellular NH_4^+ concentration is more than 50 μM, glutamine pool rises, and UTase deuridylylates GlnK and GlnB. Then, GlnK complexes with AmtB, thereby inhibiting the transport via AmtB, and ammonia may enter by diffusion. P_{II}
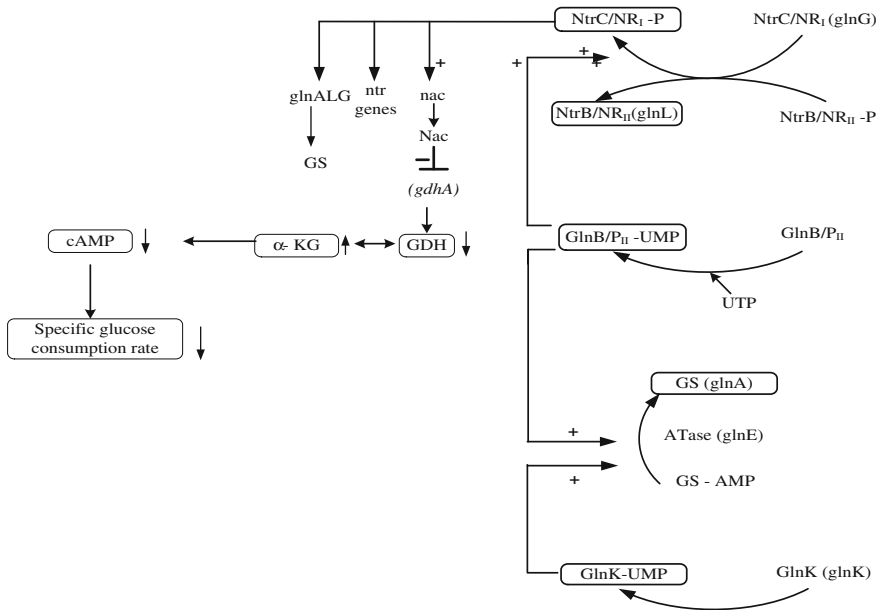
Fig. 9 Overall nitrogen regulation mechanism under N-limitation

(GlnB) interacts with NtrB (NR$_{II}$) and activates its phosphatase activity leading to dephosphorylation of NtrC (NR$_I$), and NtrC-dependent gene expression ceases (Fig. 9) [139].

Lrp regulates the expression of such genes as involved in catabolism and anabolism of amino acids (AAs). In particular, leucine indicates AA sufficiency, and it is affected by Lrp, where Lrp does not restrict to leucine but the other AAs such as isoleucine, histidine, and threonine. Lrp may activate *gltBD* and pyridine nucleotide transhydrogenase [133].

When arginine is abundant, the transcription factor ArgR binds to arginine to repress arginine biosynthesis enzymes [140] and activates arginine degradation enzymes [141]. This regulation is also subject to the NtrC regulation.

The GlnHPQ enables active transport of glutamine into the cell with higher specificity, where *glnH* is the structure gene for the periplasmic binding protein, *glnP* gene codes for the membrane-bound glutamine permease, and *glnQ* codes for the ATP hydrolyzing component of ABC transporter system [133].

Two of the major signal transduction systems of N and C metabolisms are identified as P$_{II}$ (GlnB) and PTS. Because of the important roles in the regulatory functions, P$_{II}$ and PTS can be regarded as the central processing units of N and C metabolisms, respectively. The P$_{II}$ protein senses αKG and ATP and thus links the state of central carbon and energy metabolism for the control of N assimilation [142]. N assimilation is regulated by P$_{II}$-Ntr system together with Crp, providing a regulatory network between C and N assimilation in *E. coli* [143, 144]. The C and

N metabolisms may be linked by energy metabolism, where P_{II} controls N assimilation by acting as a sensor of adenylate energy charge. Moreover, αKG serves as a cellular signal of C and N status and strongly regulates P_{II} functions [145]. Gln and αKG are the signal metabolites for nitrogen and carbon status, respectively, and these signals regulate GS adenylylation state and nitrogen regulator I (NR_I or NtrC) phosphorylation state [146]. Nitrogen shortage is reflected by the reduced Gln levels and increased αKG level [138, 147]. This ratio is substantially constant under C-limitation, where this constant ratio is the result of tight regulation of ammonia assimilation to match exactly the carbon uptake rate. This ratio is insensitive to variations in protein levels of the core circuit and to the N-utilization rate, and this robustness depends on bifunctional enzyme adenylyl transferase [148].

During N-limitation, a sudden increase in nitrogen availability results in immediate increase in glucose uptake, and αKG plays an important role for this, where αKG directly reduces the glucose uptake under N-limitation by inhibiting EI of PTS (Fig. 8) [149]. This implies the followings: (1) αKG inhibition of sugar uptake is for all PTS sugars by inhibiting EI but not carbohydrate-specific EII; (2) this is performed without perturbing the concentrations of the glycolytic intermediates such as G6P, PEP, and PYR; (3) inhibition of EI by αKG leads to reduced amount of phosphorylated $EIIA^{Glc}$ and decreases cAMP level, where the effect of αKG on cAMP production is caused by the difference in $EIIA^{Glc}$ phosphorylation rather than a difference in substrate availability [149]. Not only αKG but also other α-ketoacids such as OAA and PYR play also the similar roles and affect not only PTS but also the cAMP level by Cya [150]. Moreover, αKG is a promiscuous enzymatic regulator that competitively inhibits citrate synthase (CS) of the TCA cycle and 3PG dehydrogenase for serine biosynthesis and further controls aspartate production by product inhibition of transaminase under N-limitation [149]. αKG noncompetitively inhibits EI and Pps, while PtsP (EI homolog in the nitrogen PTS) is insensitive to αKG.

In addition to carbohydrate PTS, most proteobacteria possess a paralogous system such as nitrogen phosphotransferase system PTS^{Ntr}, where it consists of EI^{Ntr} encoded by *ptsP*, NPr encoded by *ptsO*, and $EIIA^{Ntr}$ encoded by *ptsN*, which are paralogues to the carbohydrate PTS components such as EI, HPr, and EIIA, respectively [151–153]. *E. coli* PTS^{Ntr} plays a role in relation to K^+ uptake, where dephosphorylated $EIIA^{Ntr}$ binds to and regulates the low-affinity K^+ transporter TrkA [154] and the K^+-dependent sensor kinase KdpD [153, 155]. K^+ regulates global gene expression involving both $σ^{70}$- and $σ^S$-dependent promoters [156]. Moreover, dephosphorylated $EIIA^{Ntr}$ modulates the phosphate starvation response through interaction with sensor kinase PhoR [157]. Dephosphorylated form of PTS^{Ntr} interacts with and inhibits LpxD, which catalyzes biosynthesis of lipidA of the lipopolysaccharide (LPS) layer [158].

Although the physiological role of PTS^{Ntr} has not been well known, glutamine and αKG reciprocally regulate the phosphorylation state of the PTS^{Ntr} by direct effects on EI^{Ntr} autophosphorylation. This implies that PTS^{Ntr} senses nitrogen availability [159].

9 Sulfur Regulation

Under sulfur (S) limitation, at least three metabolites such as sulfide, the reduction product of sulfate used for cysteine biosynthesis; N-acetylserine, the only precursor of cysteine; and adenosine 5'-phosphosulphate (APS), the first intermediate in sulfate assimilation, are involved for the metabolic regulation [160, 161]. Under S-limitation, the concentrations of sulfide and APS decrease, while N-acetylserine pool increases. The two regulators CysB and Cbl mediate homeostatic responses to S-limitation, where these responses help *E. coli* to scavenge trace amounts of cysteine and sulfate, preferred S sources, or the alternative S sources such as glutathione and various alkaline sulfonate including taurine. S-limitation affects methionine metabolism, synthesis of FeS clusters, and oxidative stress.

Like NtrC for N-regulation, CysB is the primary regulator for homeostatic responses to S, and it is required for the synthesis of Cbl [162]. CysB is positively controlled by N-acetylserine and negatively controlled by sulfide or thiosulfate [161], and Cbl is negatively controlled by APS [160]. It is of interest that *cbl* gene is transcribed from *nac* promoter under N-limitation [163]. The *ddp* operon is activated by NtrC, and there might be a cross-regulation between S-limitation and N-limitation [164].

10 Phosphate Regulation

The phosphate (P) metabolism is also quite important from the energy generation and phosphorelay regulation points of view. The phosphorous compounds serve as major building blocks of many biomolecules and have important roles in signal transduction [165]. Depending on the concentration of environmental phosphate, *E. coli* controls phosphate metabolism through Pho regulon, which forms a global regulatory circuit involved in a bacterial phosphate management [165, 166]. The PhoR/PhoB two-component system plays important roles in detecting and responding to the changes of the environmental phosphate concentration [167]. Namely, under phosphate limitation, the phosphate is transferred by an ABC transporter composed of PstSCAB for the high-affinity capture of P_i, and the phosphate is then transferred to PhoR (PhoR-P), and in turn, PhoB is phosphorylated by PhoR. The phosphorylated PhoB acts as the response regulator and regulates Pho Box genes such as *eda*, *phnCDEFGHIJKLMNOP*, *phoA*, *phoR/B*, *phoE*, *phoH*, *psiE*, *pstSCAB*, *phoU*, and *ugpBAECQ* [168]. When P_i is rich or in excess, P_i is taken up by the low-affinity transporter Pit, and PhoR, Pst, and PhoU together turn off the Pho regulon by dephosphorylating PhoB. The sensor protein CreC (PhoM) can phosphorylate PhoB, while acetyl phosphate can also directly phosphorylate PhoB [166]. The overall regulation mechanism is complex, and it is not so clear how the phosphate limitation affects the metabolism [169].

The promoters of the Pho genes are recognized by σ^D-associated RNA polymerase. A mutation in *rpoS* significantly increases the level of AP (alkaline phosphatase) activity, and the over-expression of σ^S inhibits it [170]. The Pho regulon is thus evolved to maintain a trade-off between cell nutrition and cell survival during P_i starvation [170].

11 Metal Ion Regulation and Oxidative Stress Regulation

Iron is ubiquitous and the fourth most abundant element on earth and supports the metabolism of living organisms on the planet [171]. Iron is involved in the formation and destruction of ROS such as superoxide ($O_2^{\cdot-}$), peroxidase (H_2O_2 and ROOH), and free radicals ($^{\cdot}OH$ and $^{\cdot}OR$) usually generated as toxic by-products of aerobic metabolism in a cascade of monovalent reductions from molecular oxygen. Although certain amounts of iron and ROS are required for the cell to survive, the excess amounts cause stress to the cell leading to the cell death [172].

The metal ion levels are often sensed by metal-sensing regulatory RNA, which encodes metal-sensing proteins involved in the transport and storage of intracellular metals [173, 174]. In the native environment, the cell continuously faces iron deficiency, where metal ion functions as cofactor in many of the cellular constituents such as flavoproteins, and therefore, the cell furnishes the mechanism for iron uptake and storage system [175, 176]. However, excess iron causes toxicity by catalyzing the formation of reactive free radicals through Fenton/Haber–Weiss reaction [177]. In combination with inability to convert NADH to NAD^+ in the respiration, a decrease in endogenous O_2^- causes reductive stress and in turn activates Fur (ferric uptake regulator) [178]. Fur generally represses ion transport and ion siderophore biosynthetic genes when complexed with ferrous ion. Under ion limitation, ion dissociates from Fur, where Fur requires binding to Fe^{2+} to become active. O_2^- deactivates Fur after its conversion to H_2O_2 by superoxide dismutase (SOD) through Fenton reaction ($H_2O_2 + Fe^{2+} \rightarrow HO^{\cdot} + OH^- + Fe^{3+}$) [179]. Therefore, a decrease in endogenous O_2^- increases the availability of Fe^{2+}, through a decrease in H_2O_2 level, and in effect activates Fur [180]. Namely, Fur senses the reductive stress and protects Fe–S clusters to be safe from damage by ROS. It is essential for the cell to use iron economically, and this is attained by siderophore synthesis and iron transport regulation [181]. Iron transport and siderophore (e.g. enterobactin) pathway genes such as *talB* and *entF* are repressed by Fur [182–184], and enterobactin may be produced in *fur* mutant *E. coli* [185]. There are functional interactions between carbon and ion utilization via Crp and Fur, where many ion transport genes and several catabolic genes are subject to dual control [186].

The widely conserved multiple antibiotic resistant regulator (MarR) family of transcription factors modulates bacterial detoxification in response to antibiotics such as fluoroquinolones and β-lactams such as ampicillin, tetracycline, and chloramphenicol, as well as toxic chemicals and synthesis of virulence

determinants. [187]. MarR senses copper (II) as a signal to cope with stress caused by antibiotics, etc., where copper (II) oxidizes a cysteine residue on MarR to generate disulfide bonds between two MarR dimers, thereby inducing tetramer formation and dissociation of MarR from its cognate promoter DNA [188].

The microbial cell responds to oxidative stress by inducing antioxidant proteins such as SOD and catalase, where those are regulated by OxyR and SoxR/S [189]. SoxR is a member of the MarR family of metal-binding transcription factors, and it exists in solution as a homodimer with each subunit containing a [2Fe–2S] cluster. These clusters are in the reduced state in inactivated SoxR, and their oxidation activates SoxR as a powerful transcription factor [190]. The active form of SoxR activates transcription of *soxS* gene, where SoxS belongs to the AraC/XylS family.

Although the respiration is universal among all aerobic organisms, inefficient electron transfer via the respiratory complexes results in one electron reduction of diatomic oxygen, a phenomenon known to generate toxic ROS [191]. Since NADPH plays an important role for detoxification of ROS, some prokaryotic microorganisms such as *E. coli* produce NADPH at ICDH in the TCA cycle together with the reactions at G6PDH, 6PGDH, and possibly at Mez.

The αKG is a key participant in the detoxification of ROS with concomitant formation of succinate, where succinate is a biomarker for oxidative stress [191]. Moreover, NADPH-producing ICDH is activated, while NADH-producing KGDH is deactivated in *Pseudomonas fluorescens* [191]. This indicates that for both prokaryotic and eukaryotic cells, the TCA cycle acts both as a scavenger and generator of ROS, and its modulation is important for regulating ROS [191]. The TCA cycle can both regulate their formation and decomposition, where the concomitant accumulation of succinate may act as a potent signal for this [191].

The proper understanding on the regulation of ROS homeostasis gives a way for medical applications [172]. Namely, iron- and ROS-dependent cell death may be considered for cancer treatment. As mentioned above for bacteria, high NADPH production with low ROS levels is essential for tumor cell proliferation and survival [192–194]. NADPH is required for glutathione homeostasis, which indicates that tumor cells require a highly reduced environment for survival. Therefore, one idea for pushing cancer cells to sentence or death is the decrease of the glutathione levels and/or the increase of the oxidative stress levels [195].

12 Redox State Regulation

Global regulators such as Fnr (fumarate nitrate reduction), Arc (anoxic respiration control) system, and Nar (nitrate reduction) are mainly responsible for the regulation under oxygen limitation and other electron acceptors in the culture environment, where Fnr directly senses molecular oxygen and plays a role under anaerobic condition [196], in coordination with ArcA/B system, where Fnr activates *arcA* gene expression. Under oxygen limitation, Fnr binds a $[4Fe–4S]^{2+}$ cluster and becomes a transcriptionally active dimeric form. Molecular oxygen can oxidize the

ion–sulfur cluster of the corresponding region, resulting in monomerization of the protein and subsequent loss of its ability to bind DNA [197]. The ArcA/B system plays a role under both anaerobic and micro-aerobic conditions [198, 199], where it is composed of ArcA, the cytosolic response regulator, and ArcB, the membrane-bound sensor kinase. The ArcA/B two-component system responds to the redox state of the membrane-associated redox carriers such as quinones in the respiratory chain [200, 201]. The quinone pool decreases under oxygen limitation and causes ArcB to be self-phosphorylated (ArcB-P), and then, ArcB-P transphosphorylates ArcA (Fig. 10) [202]. The ArcA-P then represses the expression of the TCA cycle and the glyoxylate shunt genes (Table 1). Moreover, the genes that encode the primary dehydrogenases such as *glpD*, *lctPRD, aceE,F,* and *lpdA* are also repressed by ArcA (Table 1). The *cyoABCDE* operon is repressed by both ArcA and Fnr, while *cydAB* operon is activated by ArcA and repressed by Fnr (Fig. 10) [203].

The expression of *pfl* genes which encode pyruvate formate lyase, Pfl, is activated by ArcA and Fnr, whereas *aceE,F* and *lpdA* which encode PDHc are repressed by ArcA under oxygen limitation (Fig. 10). The formate can be excreted
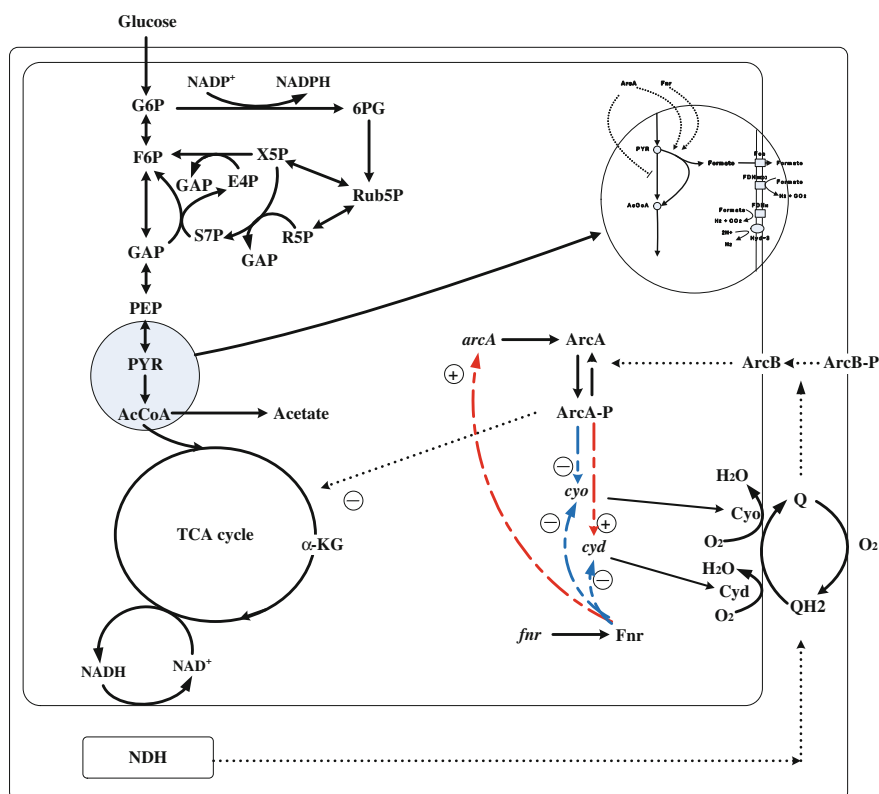

Fig. 10 Respiratory and metabolic pathway regulation by ArcA/B and Fnr

via Foc or converted to hydrogen via formate dehydrogenase, FDH_H, and formate hydrogen lyase, Fhl, and deletion of FocAB, FDH_N, and hydrogenase Hyd (Fig. 10) [204, 205]. Moreover, the flux from PYR to AcCoA is blocked in *pfl* mutants ($\Delta pflA$ or $\Delta pflB$), and pyruvate exclusively goes to lactate formation via LDH reaction [206, 207]. Moreover, Fnr activates *frd* gene expression, while repressing *sdh* gene expression, resulting in branched pathways for TCA cycle under anaerobic condition.

As mentioned before, the TCA cycle activity is repressed as the glucose consumption rate was increased due to lower level of cAMP–Crp, which in turn causes acetate overflow metabolism. This also occurs by the higher redox ratio [208]. This phenomenon can be relaxed by activating TCA cycle by *arcA/B* genes knockout [198, 204, 209]. The activated TCA cycle produces more NADH and allosterically inhibits CS and ICDH activities [210]. Thus, the NADH oxidation by the expression of *nox* gene coding for NADH oxidase, NOX, in the *arc*A mutant further reduces the acetate formation, resulting in the increased recombinant protein production [211], while nicotinic acid and Na nitrate may also activate TCA cycle [212]. The activation of the TCA cycle causes the decrease in the cell yield due to higher production of CO_2 in the TCA cycle.

Many bacteria utilize oxygen as the terminal electron acceptor, but they can switch to other acceptors such as nitrate under oxygen limitation. The reducing equivalents such as NADH are reoxidized in the respiratory chain, where oxygen, nitrate, fumarate, and dimethyl sulfoxide can be the electron acceptors. Nar plays a role when nitrate is present under oxygen limitation. Nar belongs to the two-component redox regulation systems, where it comprises a membrane sensor (NarX) that acts as a kinase causing phosphorylation of the regulator (NarL) under certain conditions [202]. The Nar system activates such genes as nitrate reduction encoding nitrate and nitrite reductases and represses such genes as *frd* genes for fumarate reductase.

13 Acid-shock Response

The cell such as *E. coli* has the regulation systems in response to acidic condition [213–216]. Some of these depend on the available extracellular amino acids such as glutamate, arginine, and lysine, where the intracellular proton is consumed by the reductive decarboxylation of the amino acid followed by the excretion of γ-amino butyric acid (GABA) from cytoplasm to the periplasm by the anti-porter that also imports the original amino acid [213]. *E. coli* is acid resistant by glutamate decarboxylase system, where *gadA* and *gadB* encode glutamate decarboxylase isozymes and *gadC* encodes glutamate/GABA anti-porter (Fig. 11). Glutamate decarboxylase is activated in response to acid, osmotic, and stationary phase signals. The GADAB forms a glutamate-dependent acid response system, where the process of decarboxylation consumes an intracellular proton and helps maintain pH homeostasis. There are other similar acid-resistant systems for the case of using
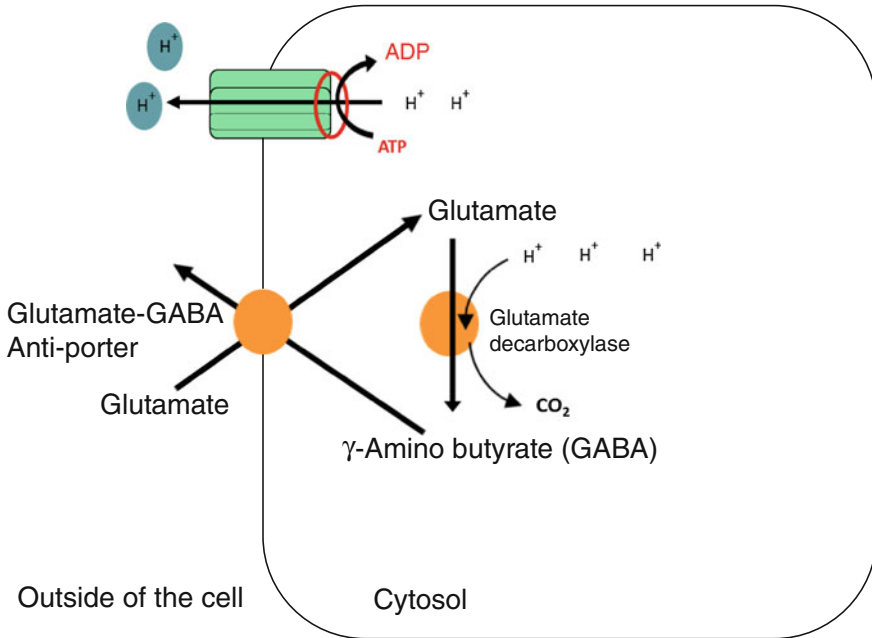
Fig. 11 Acid-shock regulation by amino acid decarboxylase and reversed PMF

arginine instead of glutamate by arginine decarboxylase, where the anti-porter is AdiC in this case [217, 218], and for the case of using lysine by lysine decarboxylase [218]. The cells grown in media rich with amino acids such as LB are acid resistant because of the above mechanism [213].

Moreover, ATPase is involved in acid regulation system [216], where ATPase is usually utilized for the protons in the periplasm move into the cytosol across the cell membrane producing ATP from ADP and P_i by the negative proton motive force (PMF). Since the basic problem of acid stress is the accumulated proton in the cytosol, this proton may be pumped out through ATPase by hydrolyzing ATP with reversed proton move due to positive PMF at low pH such as pH 2 or 3 [216]. Without amino acid in the media, this acid response system is activated by utilizing ATPase [215, 219], where the positive PMF pumps out extra protons (H^+) from the cytoplasm using ATP (Fig. 11) [215]. This proton homeostasis by PMF is conserved in large class of organisms.

RpoS that increases at the late growth phase and the stationary phase and Crp are involved in acid resistance [213, 220]. As implied by the involvement of Crp, the acid-resistant system is repressed when glucose is present. The acidic pH lowers cAMP levels in exponentially growing cells in the minimal glucose medium. Since cAMP–Crp represses RpoS, this may elevate RpoS and increases the expression of *gadX*. The overall regulation system seems to be quite complex involving EvgS/A, B1500, PhoQ/PhoP, GadX, GadW, etc. [221].

OmpR may be a key regulator for acid adaptation, and thus, *ompR* mutant is sensitive to acid exposure [222]. The acid-inducible *asr* gene is regulated by PhoR/B, and thus, *phoR/phoB* deletion mutant fails to induce *asr* gene expression [223].

In order to keep pH constant, alkali such as NaOH is supplied during the cell growth in practice, which results in the increase in sodium ion (Na^+), where *nhaA* gene encoding the Na^+/H^+ anti-porter membrane protein and *nhaR* gene encoding the NhaA regulatory protein can be over-expressed in *pflB* mutant, showing performance improvement for lactate fermentation [224].

14 Heat-shock Stress Response

The organisms respond to a sudden temperature upshift by increasing the synthesis of a set of proteins. This phenomenon is called as heat-shock response, where this does not restrict to the temperature upshift, but also other stresses such as solvent stress. The heat-shock proteins play important roles in the assembly and disassembly of macromolecular complex such as GroE, the intracellular transport such as Hsp70, transcription such as σ^{70}, proteolysis such as Lon, and translation such as lysyl tRNA synthetase. The heat-shock response in *E. coli* is mediated by σ^{32} encoded by *rpoH*. Among them, *groEL, dnaK*, and *htpG* encode major chaperones such as Hsp 60, Hsp 70, and Hsp 90. ClpP, Lon, and HtrC are involved in the proteolysis. DnaK, DnaJ, GrpE, and RpoH are involved in the autoregulation of heat-shock response. DnaK prevents the formation of inclusion bodies by reducing aggregation and promotion of proteolysis of misfolded proteins. A bichaperone system involving DnaK and ClpB mediates the solubilization or disaggregation of proteins. GroEL operates protein transit between soluble and insoluble protein fractions and participates positively in disaggregation and inclusion body formation. Small heat-shock proteins such as IbpA and IbpB protect heat-denatured proteins from irreversible changes in association with inclusion bodies [225, 226].

Hoffmann et al. [227] investigated the metabolic adaptation of *E. coli* during temperature-induced recombinant protein production and showed that cAMP–Crp-controlled LpdA of pyruvate dehydrogenase complex (PDHc) and SdhA in the TCA cycle are highly induced. Namely, the TCA cycle is activated due to increased level of cAMP–Crp at higher temperature. In *E. coli*, heat-shock protein synthesis rates peak at about 5–10 min after the temperature upshift and then decline to a new steady-state level [228]. The σ^{70} is itself a heat-shock protein, and the increase in its concentration after heat shock may contribute to its decline in heat-shock protein synthesis. DnaK contributes to the shutoff of the high-level synthesis of heat-shock proteins [229]. The heat shock activates *crp* gene expression, and in turn, Crp activates *mlc* gene which codes for Mlc [230], and thus, the glucose consumption rate decreases (Fig. 12) [231]. This also causes cAMP level to be increased (Fig. 12).

Acetate production is affected by higher temperature. Transcription of *acs* gene occurs from two σ^{70}-dependent promoters such as distal promoter *acs* P_1 and proximal promoter *acs* P_2 [232, 233]. The cAMP–Crp binds two sites within the *acs*
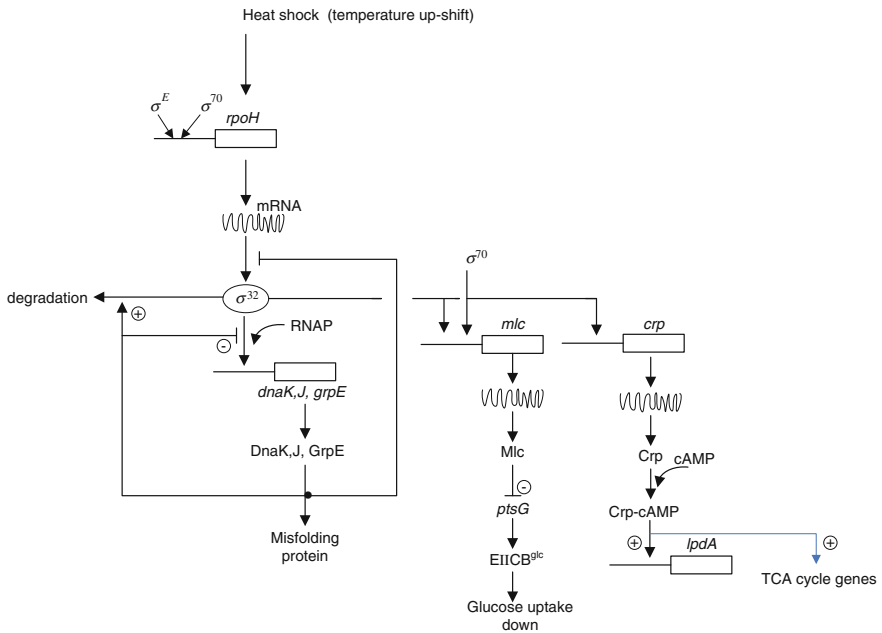
Fig. 12 Overall heat-shock regulation mechanism

regulatory region. However, Fis and Ihf independently modulate Crp-dependent activation of *acs* P_2 transcription [234, 235].

The respiration is activated during the temperature upshift [227], and *sod* is induced in response to the oxidative stress imposed by dioxygen or by the redox-active compounds such as viologens or quinones caused by the temperature upshift [236]. This phenomenon may be also caused by the activated TCA cycle.

15 Cold-shock Response

Upon temperature downshift from 37 to 15 °C, the major cold-shock proteins such as CspA, CspB, and CspG are induced, where cold-shock proteins are able to bypass the inhibitory effect of the antibiotics such as kanamycin and chloramphenicol [237]. Although thermoregulatory mechanism is not well understood, the adaptation of the cell to low temperature such as 20–23 °C requires coordinated and multifunctional response, where RpoS and the small regulatory RNA DsrA are involved in both cold-shock and biofilm formation genes [238] as well as flagella biosynthesis and motility genes [239].

16 Solvent Stress Regulation

The biofuels production by microorganisms has been paid recent attention. However, many biofuels are toxic to microorganisms and reduce the cell viability through damage to the cell membrane and interference with essential physiological processes. Several attempts have been made to improve the tolerance to biofuels, where biofuel export systems, heat-shock proteins, and membrane modifications have been considered [240]. The effect of biofuels on the cell is through hydrophobicity of the cytoplasmic membrane, where the accumulation of solvent in the cytoplasmic membrane increases permeability of membrane, diminishes energy transduction, interferes with membrane protein function, and increases fluidity [240–243]. This may cause the release of ATP, ions, phospholipids, RNA, and proteins, and thus, the cell growth is depressed due to disturbances on ATP production by diminished PMF. Moreover, the increase in fluidity affects the nutrient transport as well as energy transduction.

Toxicity levels vary depending on the microbes and the types of biofuels and biochemicals. In general, longer chain alcohols are more toxic than short-chain alcohols. Efflux pumps are membrane transporters that recognize and export toxic compounds from the cell by PMF, where this is important for the cell to survive by exporting bile salts, antimicrobial drugs, and solvents. The *acrAB–tolC* pump in *E. coli* provides tolerance to hexane, heptanes, octane, and nonane [244]. Efflux pumps are effective for increasing tolerance and production of biofuels, in particular, for long-chain alcohols, but those are not effective for exporting short-chain alcohols such as 1-propanol and isobutanol [245].

The heat-shock proteins are up-regulated in response to short-chain alcohols [180, 246], and heat-shock protein refolding genes such as *rpoH, dnaJ, htpG,* and *ibpAB* are up-regulated [247], while *groESL, dnaKJ, hsp18, and hsp90* are up-regulated in *Chrostridium acetobutylicum* [248]. Over-expression of heat-shock proteins may increase tolerance against biofuels [249, 250].

In general, solvents disrupt the cell membrane structure and have a strong impact on physiological function and eventually leading to the cell death [251]. To overcome this problem, solvent-tolerant microbes change the composition of the fatty acids from *cis* to *trans* unsaturated fatty acids catalyzed by *cis–trans* isomerase (*cti*), thus decreasing membrane fluidity, preventing the entry of solvents into the cell [252, 253]. In addition, modifications to phospholipid headgroups or phospholipid chain length increase solvent tolerance [246].

In relation to solvent stresses caused by the accumulation of biofuels in the culture broth, the primary role to protect the cell from such stress is made by outer membrane porin proteins. Since cytosolic membrane is also under stress condition, respiration and membrane proteins as well as general stress response mechanism are affected [243]. ROS highly increase in response to the stress caused by n-butanol in *E. coli* [247].

17 Osmoregulation

The bacterial cell is surrounded by the cell envelope, where the plasma membrane is responsible for the transport of ions such as H^+, Na^+, and K^+, and various substrates or nutrients, and metabolites to maintain homeostasis. The bacterial cells exchange such components together with energy and information with their surroundings by the appropriate sensing and responding mechanisms [254]. Under osmotic stress condition, a number of transport systems for ions such as K^+, and compatible solutes such as proline betaine and the precursor choline are activated [255]. The typical two-component histidine kinase/response regulator system such as KdpD/KdpE is ubiquitous in various bacteria [256], where it regulates *kdpFABCDE* operon including the Kdp ATPase and active K^+ uptake system. Namely, KdpD/KdpE system responds to K^+ limitation and salt stress [257–259]. As also mentioned before, EnvZ/OmpR two-component system regulates the expression of the porin genes such as *ompC* and *ompF* encoding outer membrane porins in relation to osmolarity.

The cytoplasmic or inner membrane is impermeable to most large and poler solutes, while these are compensated for by freely diffusing water molecules, and thus, the transmembrane concentration gradients are developed for such compounds. The resulting changes in cellular volume and turgor pressure exert strong mechanical force on the cytoplasmic membrane and associated proteins and preclude the cell growth [260]. To cope with osmotic stress, bacteria adapt their intracellular osmolarity [254] or increase the cell wall stability [261]. The salt stress tolerance is mediated by flux control of water across the cell membrane, adjustments of intracellular potassium levels, synthesis of disaccharide trehalose, and/or transport of small molecule osmoprotectants [262].

In principle, bacterial cells respond to environmental or growth conditions by immediate protein or enzyme-level regulation, and by slow gene transcriptional regulation via transcription factors. In summary [260], in response to sudden changes in osmotic pressure, *E. coli* controls in- and outflux of water and other small molecules by activating aquaporins as an immediate response [263]. It regulates intracellular potassium concentrations by adjusting the potassium transporters such as Kup, KdpFABC, or TrkA for transient adaptation to short-term osmotic stress [264]. In the case of prolonged osmotic stress, *E. coli* takes up the osmotolerants such as glycine betaine and proline from the environment via ABC transporter encoded by *proVWX* or synthesizes glycine betaine from the extracellular precursor choline [265–267]. If no extracellular compatible solutes are available, *E. coli* induces expression of trehalose 6-phosphate synthase (OtsA) and phosphatase (OtsB) to produce high intracellular concentrations of the nonreducing disaccharide trehalose from the precursors such as UDP-glucose and G6P in response to long-term resistance to sustained osmotic stress [268–270].

There is indeed an interaction between trehalose and membrane lipid head groups, but this effect is insufficient to fully account for the resistance of membrane against strong osmotic stress. Upon osmotic stress, bacteria adjust their intracellular
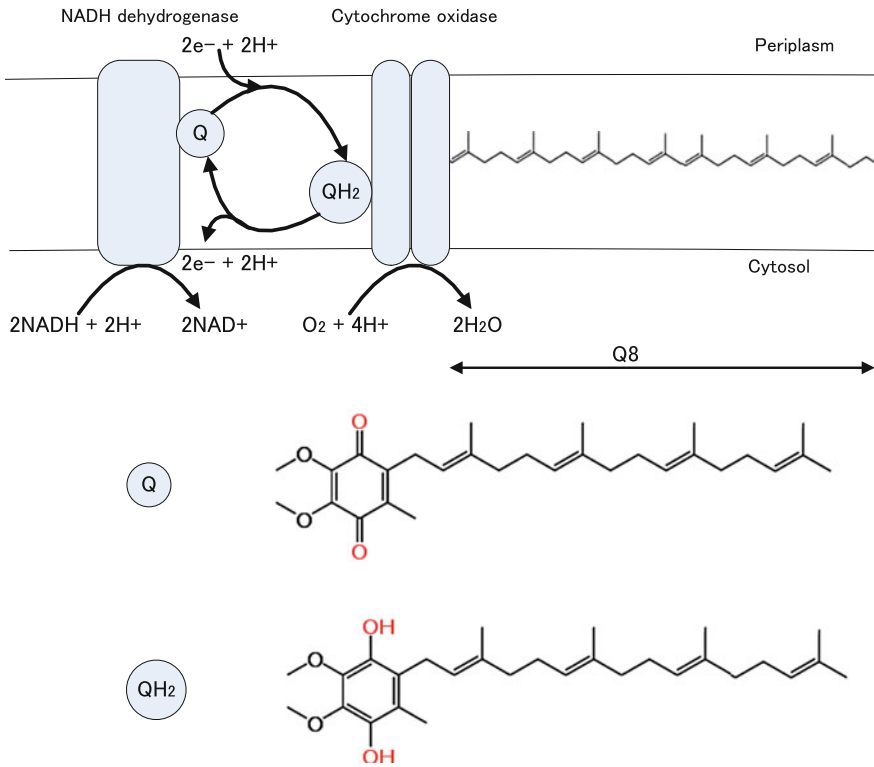
Fig. 13 Electron transport carrier quinone and quinol in the respiratory chain and the role of osmoprotection

osmolarity and modify their cell wall structure [260]. For this, polyisoprene lipids may also contribute to osmoprotection by increasing resistance to high-salt conditions in the cytoplasmic membrane and in the membrane bilayers of liposomes in *E. coli* [260]. Coenzyme Q functions as an electron and proton carrier in aerobic respiration and has an additional crucial role as a chain-breaking antioxidant [271]. The long polyisoprenyl tail of CoQ_n functions to anchor this lipid in the membranes of cells (Fig. 13), where n designates the number of five carbon isoprene units such as CoQ_6 in *S. cerevisiae*, CoQ_8 in *E. coli*, and CoQ_{10} in human [262]. In *E. coli*, CoQ_8 level becomes significantly high in response to high-salt condition (Fig. 13) [260].

## 18 Biofilm, Motility by Flagella, and Quorum Sensing

Biofilm formation is one of the important microbial survival strategies, where biofilm development involves attachment of bacteria to surfaces and cell–cell adhesion to form microcolonies. This is useful for the cell to protect against

predators and antibiotics [272]. The attachment of bacteria to abiotic and biotic surfaces is made by motility, proteinaceous adhesion, and a cell-bound polysaccharide such as PGA (poly-β-1,6-N-acetyl-D-glucosamine), where PGA is a cell-bound exopolysaccharide adhesion [272]. As mentioned before, Csr plays important roles for biofilm formation, where *pga* operon involved in PGA formation and excretion is negatively regulated by CsrA. CsrA also negatively regulates c-di-GMP, a second messenger involved in biofilm formation and motility [273]. Curli are extracellular proteinaceous structures extending from the cell surface for attachment during biofilm development [274]. Curli filaments are activated by CsgD, where it is inversely correlated with flagella synthesis. The master regulator of flagella synthesis is $FlhD_2C_2$, which activates the genes involved in motility and chemotaxis [275]. McaS (multicellular adhesion sRNA) represses CsgD expression, while activates FlhD and PgaA [275], and thus regulates the synthesis of curli flagella and polysaccharide. Moreover, biofilm formation is under catabolite repression by cAMP and Crp [276].

Quorum sensing is a cell-to-cell communication [277], where the signal molecules are homoserine lactones (AHL) synthesized by LuxI-type enzyme. At high cell density cultivations, LuxR-type regulator plays a role for the positive feedback in association with AHL when its concentration exceeds a threshold level [120]. The quorum sensing is the sensing of cell density, where in *E. coli*, CyaR represses *luxS* gene which encodes autoinducer-2 synthase [278].

19 Systems Biology Approach

In order to deepen our knowledge on metabolic regulation and for the efficient metabolic engineering, it is quite useful to develop the appropriate metabolic models which describe the dynamic behavior of the intracellular metabolite concentration [279, 280]. Although kinetic models for the glycolysis and the PP pathways have been developed for *E. coli* [281], it is better to include TCA cycle, thus covering the whole main metabolism, which enables the simulation of the aerobic batch and continuous cultivations. Since the fluxes of the main metabolic pathways can be computed with respect to time by such a model, the cell growth rate may be reasonably predicted by taking into account the experimental observation that the cell growth rate is correlated with the specific ATP production rate [282]. More importantly, it is highly desirable to incorporate the effects of transcription factors on the enzymatic reactions to simulate the transition of the metabolism during the batch culture [42, 283, 284]. This type of model can be used to simulate acetate overflow metabolism and co-consumption of multiple sugars in relation to catabolite regulation [285]. As shown in Fig. 14, the trend of the transcription factors such as cAMP–Crp and Cra, and the pathway activities can be well predicted with such a model for the case of continuous culture as mentioned in Sect. 3, and it is quite useful to understand the complicated metabolic regulation mechanism [285].
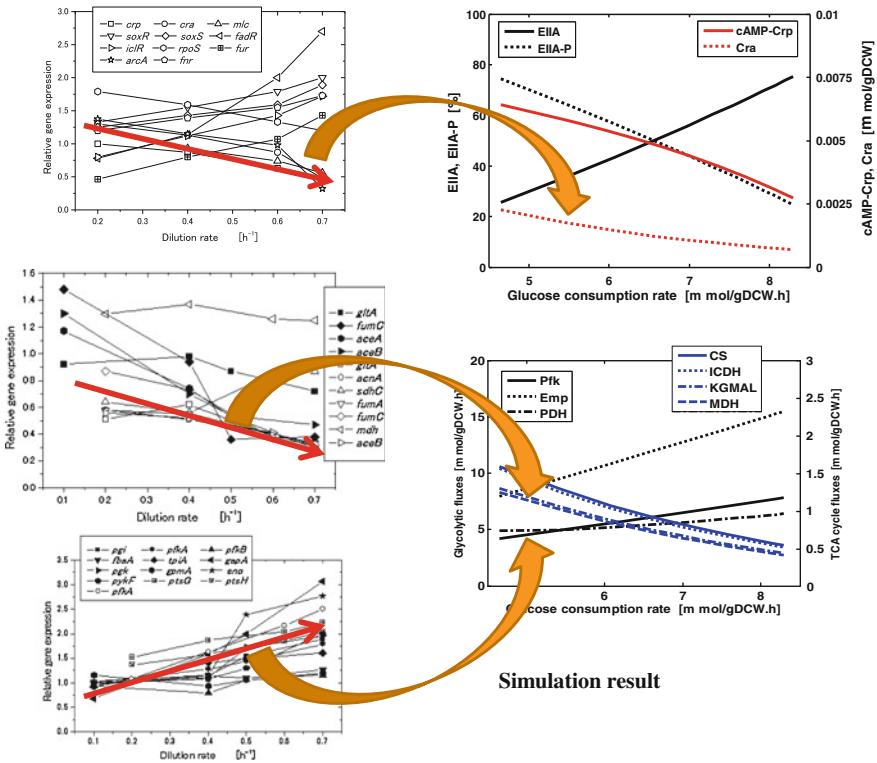
Fig. 14 Comparison of simulation result [45] with experimental data [48] for the continuous culture

20 Concluding Remarks

As seen above, the global regulators are responsive to the specific stimuli. Examples of such pleiotropic TFs in *E. coli* are Crp, a primary sensor for C-availability; NtrBC, a sensor for N-availability; PstSCAB and PhoR, the sensor for P-availability; CysB, the sensor for S-availability; and Fur, the sensor for ion availability. Functional interactions among such regulators must coordinate the activities of the metabolon so that the supply of one type of nutrient matches the supply of other nutrients [286]. Thus, multiple links between C and N metabolism have been identified [287]. Other functional links between C and S metabolism [288], and between C and ion metabolism [289, 290] have been identified. Moreover, the links between S and N limitations have been also identified [291].

In general, bacteria in nature live far away from the optimal growth condition, where multiple stresses are imposed on the cell. Therefore, the cell must have the ability to sense, integrate, and respond to the variety of stresses for survival.

Although little is known about "cross-stress" protection, cross-stress dependencies are ubiquitous, highly interconnected and may emerge within short time frames [292]. In fact, high degree of overlap was observed in the transcriptional profiling for different stresses such as starvation, osmotic, and acidic stresses [293], as well as starvation and heat-shock or oxidative stress [294, 295], where high osmolarity and high temperature induce the oxidative stress regulons such as SoxRS and OxyR [296, 297]. The responses to n-butanol share the same high overlap with those in heat-shock, oxidative, and acidic stresses [298].

As mentioned in this article, the specific metabolites such as FBP, PEP, PYR, OAA, AcCoA, and αKG in the main metabolic pathways play important roles for metabolic regulation. This implies that these metabolites play roles for the coordinated and integrated metabolic regulation. The regulation system ranges from relatively rapid interactions such as enzyme-level regulation by allosteric binding of the specific molecules or posttranslational modification to slow interactions such as transcriptional regulation via transcription factors. It is important to get deep insight into the whole cellular metabolic systems not only by molecular biology and biochemistry, but also by systems biology approach, and apply this for the efficient metabolic engineering.

Among intracellular metabolites, α-ketoacids such as αKG, OAA, and PYR turn to be mater regulators for catabolite regulation and coordination of different regulations [299]. Namely, when favoured carbon sources are depleted, α-ketoacid levels fall, and cAMP increases to stimulate other carbon catabolite machinery. When preferred nutrients are abundant, the cell growth rate becomes higher with lower cAMP level, while if they are scarce, the cell growth rate declines with higher cAMP level. This change in growth rate is accompanied by a change in cellular composition, where ribosomes are needed for rapid protein production at higher growth rate, while more metabolic enzymes for nutrient assimilation (catabolism) are needed at lower cell growth rate [300, 301]. There is a linear relationship between the total protein composition of a cell and its growth rate, where this can be extended beyond ribosomes to metabolic enzymes [150]. Under N- or S-limitation or other nutrient limitation, α-ketoacids such as αKG accumulate and inhibit carbon assimilation, where there is less need for carbon catabolic enzymes and more demand for those involved in such nutrient assimilation. When anabolic nutrients are in excess, αKG concentration decreases, cAMP level increases, and carbon catabolic enzymes increase to accelerate carbon assimilation. In the end, the physiological function of cAMP signaling goes beyond simply enabling hierarchical utilization of carbon sources, but also controls the function of the proteome [150, 299]. The energy level also affects carbon uptake rate [20, 302].

As mentioned before, cAMP–Crp and Cra play important roles for carbon catabolite regulation in *E. coli*, where either PTS or non-PTS sugars are ranked for assimilation in a hierarchy [303–305]. This may be caused by the cAMP–Crp level and the promoter activities of the corresponding promoters of the transporters [303–305].

The roles of cAMP–Crp are not only limited to carbon catabolite regulation. Among many transcription factors, Crp plays significantly important roles in the

wide range of regulations such as osmoregulation [306], osmotolerance [307], oxidative stress [308], acid tolerance [309], acetate tolerance [310], ethanol tolerance [311], and butanol tolerance [312] as well as catabolite regulation. Therefore, it may be of interest to modulate such transcription factors [313, 314] for the development of next-generation cell factories.

It is of surmount interest to understand how the cell growth rate is regulated, since such information gives us a hint for improving the cell growth rate, and thus increasing the protein or metabolic production. For this, it is important to recognize at which regulation levels affect the cell growth rate, where posttranscriptional control of protein abundances and posttranslational control of flux rates are dominated [315].

Moreover, it is also important to understand the effect of the specific pathway gene mutation on the metabolic regulation in addition to the effect of growth condition. In fact, ^{13}C-metabolic flux analysis has been extensively employed for the metabolic flux distributions of pathogens such as *Mycobacterium tuberculosis* [316] and the specific gene knockout mutant *E. coli* [48, 317, 318], where the flux information is located on top of different levels of information, manifested as the result of metabolic regulation, and central to understanding the metabolism. It is, therefore, of interest to investigate the effect of the specific gene knockout on the metabolism as well in view of metabolic regulation for the design of next-generation cell factories.

References

Introduction

1. Jone RG, Thompson CB (2009) Tumor suppressors and cell metabolism: a recipe for cancer growth. Genes Dev 23:537–548
2. Hsu PP, Sabatini DM (2008) Cancer cell metabolism: Warburg and beyond. Cell 134:703–707
3. Kroemer G, Pouyssegur J (2008) Tumor cell metabolism: cancer's Achilles' heel. Cancer Cell 13:472–482
4. Folger O et al (2011) Predicting selective drug targets in cancer through metabolic networks. Mol Syst Biol 7:501
5. McInerney MJ, Sieber JR, Gunsalus RP (2009) Syntrophy in anaerobic global carbon cycles. Curr Opin Biotechnol 20:623–632
6. Dolfing J, Jiang B, Henstra AM, Stams AJ, Plugge CM (2008) Syntrophic growth on formate: a new microbial niche in anoxic environments. Appl Environ Microbiol 74:6126–6131
7. Lin LH et al (2006) Long-term sustainability of a high-energy, low-diversity crustal biome. Science 314:479–482
8. Peralta-Yahya PP, Zhang F, del Cardayre SB, Keasling JD (2012) Microbial engineering for the production of advanced biofuels. Nature 488:320–328

9. Atsumi S, Hanai T, Liao JC (2008) Non-fermentative pathways for synthesis of branched-chain higher alcohols as biofuels. Nature 451:86–90

10. Yim H et al (2011) Metabolic engineering of *Escherichia coli* for direct production of 1,4-butanediol. Nat Chem Biol 7:445–452

11. Dellomonaco C, Clomburg JM, Miller EN, Gonzalez R (2011) Engineered reversal of the β-oxidation cycle for the synthesis of fuels and chemicals. Nature 476:355–359

12. Bond-Watts BB, Bellerose RJ, Chang MC (2011) Enzyme mechanism as a kinetic control element for designing synthetic biofuel pathways. Nat Chem Biol 7:222–227

13. Shen CR et al (2011) Driving forces enable high-titer anaerobic 1-butanol synthesis in *Escherichia coli*. Appl Environ Microbiol 77:2905–2915

14. Keasling JD (2010) Manufacturing molecules through metabolic engineering. Science 330:1355–1358

15. Shimizu K (2014) Biofuels and biochemicals production by microbes. NOVA Publishing Co., USA

16. Bar-Even A, Flamholz A, Noor E, Mil R (2012) Rethinking of glycolysis: on the biochemical logic of metabolic pathways. Nat Chem Biol 8:509–517

17. Flamholz A, Noor E, Bar-Even A, Liebermeister W, Milo R (2013) Glycolytic strategy as a tradeoff between energy yield and protein cost. PNAS USA 110(24):10039–10044

18. Shimizu K (2013) Metabolic regulation of a bacterial cell system with emphasis on *Escherichia coli* metabolism. *ISRN Biochemistry,* doi:10.1155/2013/645983 (Article ID 645983)

19. Shimizu K (2014) Metabolic regulation of *Escherichia coli* in response to nutrient limitation and environmental stress conditions. Metabolites 4:1–35

20. Chubukov V, Gerosa L, Kochanowski K, Sauer U (2014) Coordination of microbial metabolism. Nat Rev 12:327–340

Transport of Nutrient and Waste

21. de la Cruz MA, Fernandez-Mora M, Guadarrama C et al (2007) LeuO antagonizes H-NS and StpA-dependent repression in *Salmonella enterica* ompS1. Mol Microbiol 66(3):727–743

22. Nikaido H (2003) Molecular basis of bacterial outer membrane permeability revisited. Microbiol Mol Biol Rev 67(4):593–656

23. Nikaido H, Vaara M (1985) Molecular basis of bacterial outer membrane permeability. Microbiol Rev 49(1):1–32

24. Death A, Ferenci T (1994) Between feast and famine: endogenous inducer synthesis in the adaptation of *Escherichia coli* to growth with limiting carbohydrates. J Bacteriol 176(16):5101–5107

25. Gunnewijk MGW, van den Bogaard PTC, Veenhoff LM et al (2001) Hierarchical control versus autoregulation of carbohydrate utilization in bacteria. J Mol Microbiol Biotechnol 3(3):401–413

26. Poolman B, Konings WN (1993) Secondary solute transport in bacteria. Biochim Biophy Acta 1183(1):5–39

27. Deutscher J, Francke C, Postoma PW (2006) How phosphotransferase system-related protein phosphorylation regulates carbohydrate metabolism in bacteria. Microbiol Mol Biol Rev 70(4):939–1031

28. Palm K, Luthman K, Ros J, Grasjo J, Artursson P (1999) Effect of molecular charge on intestinal epithelial drug transport: pH-dependent transport of cationic drugs. J Pharmacol Exp Ther 291:435–443

29. Chakrabarti AC, Deamer DW (1992) Permeability of lipid bilayers to amino acids and phosphate. Biochim Biophys Acta 1111:171–177

30. Chakrabarti AC, Deamer DW (1994) Permeation of membranes by the neutral form of amino acids and peptides: relevance to the origin of peptide translocation. J Mol Evol 39:1–5

31. Finkelstein A (1976) Water and nonelectrolyte permeability of lipid bilayer membranes. J Gen Physiol 68:127–135
32. Winiwarter S et al (1998) Correlation of human jejunal permeability (in vivo) of drugs with experimentally and theoretically derived parameters. A multivariate data analysis approach. J Med Chem 41:4939–4949
33. Davis BD (1958) On the importance of being ionized. Arch Biochem Biophys 78:497–509
34. Westheimer FH (1987) Why nature chose phosphates. Science 235:1173–1178

PTS and Carbon Catabolite Regulation

35. Gorke B, Stulke J (2008) Carbon catabolite repression in bacteria: many ways to make the most out of nutrients. Nat Rev Microbiol 6:613–624
36. Inada T, Kimata K, Aiba H (1996) Genes Cells 1:293–301
37. Gosset G (2005) Improvement of *Escherichia coli* production strains by modification of the phosphoenolpyruvate: sugar phosphotransferase system. Microb Cell Fact 4:14
38. Hogema BM, Arents JC, Bader R, Eijkemans K et al (1998) Inducer exclusion in *Escherichia coli* by non-PTS substrates: the role of the PEP to pyruvate ratio in deter-mining the phosphorylation state of enzyme IIA^{Glc}. Mol Microbiol 30:487–498
39. Bettenbrock K, Sauter T, Jahreis K, Klemling A, Lengeler JW, Gilles ED (2007) Correlation be-tween growth rates, EIIACrr phosphorylation, and intra-cellular cyclic AMP levels in *Escherichia coli* K-12. J Bacteriol 189:6891–6900
40. Schaub J, Reuss M (2008) In vivo dynamics of glycolysis in *E. coli* shows need for gowth-rate dependent metabolome analysis. Biotechnol Prog 24:1402–1407
41. Kochanowski K, Volkmer B, Gerosa L, van Rijsewijk HBR, Schmidt A, Heinemann M (2013) Functioning of a metabolic flux sensor in *Escherichia coli*. PNAS USA 110:1130–1135
42. Kotte O, Zaugg JB, Heinemann M (2010) Bacterial adaptation through distributed sensing of metabolic fluxes. Mol Syst Biol 6:355
43. Huberts DH, Niebel B, Heinemann M (2012) A flux-sensing mechanism could regulate the switch between respiration and fermentation. FEMS Yeast Res 12(2):118–128
44. Kremling A, Bettenbrock K, Gilles ED (2008) A feed-forward loop guarantees robust behavior in *Escherichia coli* carbohydrate uptake. Bioinformatics 24:704–710
45. Matsuoka Y, Shimuzu K (2013) Catabolite regulation analysis of *Escherichia coli* for acetate overflow mechanism and co-consumption of multiple sugars based on systems biology approach using computer simulation. J Biotechnol 168:155–173
46. Shimada T, Yamamoto K, Ishihama A (2011) Novel members of the Cra regulon involved in carbon metabolism in *Escherichia coli*. J Bacteriol 193(3):649–659
47. Saier MH Jr, Ramseier TM (1996) The catabolite repressor/activator (Cra) protein of enteric bacteria. J Bacteriol 178:3411–3417
48. Ishii N, Nakahigashi K, Baba T, Robert M, Soga T, Kanai A, Hirasawa T, Naba M, Hirai K, Hoque A, Ho PY, Kakazu Y, Sugawara K, Igarashi S, Harada S, Masuda T, Sugiyama N, Togashi T, Hasegawa M, Takai Y, Yugi K, Arakawa K, Iwata N, Toya Y, Nakayama Y, Nishioka T, Shimizu K, Mori H, Tomita M (2007) Multiple high-throughput analyses monitor the response of *E. coli* to perturbations. Science 316:593–597
49. Valgepea K, Adamberg K, Nahku R, Lahtvee PJ, Arike L, Vilu R (2010) Systems biology approach reveals that overflow metabolism of acetate in *Escherichia coli* is triggered by carbon catabolite repression of acetyl-CoA synthetase. BMC Syst Biol 4:166
50. Yao R, Hirose Y, Sarkar D, Nakahigashi K, Ye Q, Shimizu K (2011) Catabolic regulation analysis of *Escherichia coli* and its *crp*, *mlc*, *mgsA*, *pgi* and *ptsG* mutants. Microb Cell Fact 10:67

51. Wolf RE, Prather DM, Shea FM (1979) Growth-rate dependent alteration of 6-phosphogluconate dehydrogenase and glucose 6-phosphate dehydrogenase levels in *Escherichia coli* K-12. J Bacteriol 139:1093–1096

Acetate Overflow Metabolism and Reduction of Acetate Formation in *E. coli*

52. Wong MS, Wu S, Causey TB, Bennet GN, San KY (2008) Reduction of acetate accumulation in *Escherichia coli* cultures for increased recombinant protein production. Metab Eng 10:97–108
53. Russel JB et al (2007) The energy spilling reactions of bacteria and other organisms. J Mol Microbiol Biotechnol 13:1–11
54. Valgepea K, Adamberg K, Vilu R (2011) Decrease of energy spilling in *Escherichia coli* continuous cultures with rising specific growth rate and carbon wasting. BMC Syst Biol 5:106
55. Kayser A, Weber J, Hecht V, Rinas U (2005) Metabolic flux analysis of *Escherichia coli* in glucose-limited continuous culture. I. Growth-rate dependent metabolic efficiency at steady state. Microbiology 151:693–706
56. Wolfe AJ (2005) The acetate switch. Microbiol Mol Biol Rev 69:12–50
57. Majewski RA, Domach MM (1990) Simple constrained-optimization view of acetate overflow in *Escherichia coli*. Biotech Bioeng 35:732–738
58. Yang C, Hua Q, Baba T, Mori H et al (2003) Analysis of *E. coli* anaplerotic metabolism and its regulation mechanism from the metabolic responses to alter dilution rates and *pck* knockout. Biotechnol Bioeng 84:129–144
59. Lee SY (1996) High cell-density cultivation of *Escherichia coli*. Trends Biotechnol 14:98–105
60. Hardiman T, Lemuth K, Keller MA, Reuss M, Siemann-Herzberg M (2007) Topology of the global regulatory network of carbon limitation in *Escherichia coli*. J Biotechnol 132:359–374
61. Konstantinov K, Kishimoto M, Seki T, Yoshida T (1990) A balanced DO-stat and its application to the control of acetic acid excretion by recombinant *Eshcherichia coli*. Biotechnol Bioeng 36(7):750–758
62. Ferreira AR, Ataide F, von Stosch M, Jias JML et al (2012) Application of adaptive DO-stat feeding control to *Pichia pastoris* X33 cultures expressing a single chain antibody fragment (scFv). Bioprocess Biosyst Eng 35(9):1603–1614
63. Li K-T, Liu D-H, Chu J, Wang Y-H et al (2008) An efficient and simplified pH-stat control strategy for the industrial fermentation of vitamin B12 by *Pseudomonas denitrificans*. Bioprocess Byosyst Eng 31:605–610
64. Pirt SJ (1982) Maintenance energy: a general model for energy-limited and energy-sufficient growth. Arch Microbiol 133:300–302
65. Hewitt, CJ, Caron NV, Nienow AW, McFarlane CM (1999) Use of multi-staining flow cytometry to characterise the physiological state of *Escherichia coli* W3110 in high cell density fed-batch cultures. *Biotechnol Bioeng* 63:705–711
66. Hewitt CJ, Caron NV, Axelsson B, McFarlane CM, Nienow AW (2000) Studies related to the scale-up of high-cell-density *E. coli* fed-batch fermentations using multiparameter flow cytometry: effect of a changing microenvironment with respect to glucose and dissolved oxygen concentration. *Biotechnol Bioeng* 70(4):381–390
67. Hewitt CJ, Nebe-Von-Caron G (2001) An industrial application of multiparameter flow cytometry: Assessment of cell physiological state and its application to the study of microbial fermentations. Cytometry 44:179–187

68. Peebo K, Valgepea K, Nahku R, Riis G, Õun M, Adamberg K, Vilu R (2014) Coordinated activation of PTA-ACS and TCA cycles strongly reduces overflow metabolism of acetate in *Escherichia coli*. Appl Microbiol Biotechnol, doi: 10.1007/s00253-014-5613-y

Catabolite Regulation for the Uptake of Various Carbon Sources

69. Vasudevan P, Briggs M (2008) Biodiesel production–current state of the art and challenges. J Ind Microbiol Biotechnol 35:421–430
70. Dharmadi Y, Murarka A, Gonzalez R (2006) Anaerobic fermentation of glycerol by *Escherichia coli*: a new platform for metabolic engineering. Biotechnol Bioeng 94:821–829
71. Clomburg JM, Gonzalez Ramon (2013) Anaerobic fermentation of glycerol: a platform for renewable fuels and chemicals. Trends Biotechnol 31(1):20–28
72. Almeida JRM, Fávaro LCL, Betania F, Quirino BF (2012) Biodiesel biorefinery: opportunities and challenges for microbial production of fuels and chemicals from glycerol waste. Biotechnol Biofuels 5:48
73. Martínez-Gómez K, Flores N, Castañeda HM, Martínez-Batallar G, Hernández-Chávez G, Ramírez OT, Gosset G, Encarnación S, Bolivar F (2012) New insights into *Escherichia coli* metabolism: carbon scavenging, acetate metabolism and carbon recycling responses during growth on glycerol. Micob Cell Fact 11:46
74. Peng L, Shimizu K (2003) Global metabolic regulation analysis for *Escherichia coli* K12 based on protein expression by 2-dimensional electrophoresis and enzyme activity measurement. Appl Microbiol Biotechnol 61:163–178
75. Oh MK, Liao JC (2000) Gene expression profiling by DNA microarrays and metabolic fluxes in *Escherichia coli*. Biotechnol Prog 16:278–286
76. Applebee MK, Joyce AR, Conrad TM, Pettigrew DW, Palsson BO (2011) Functional and metabolic effects of adaptive glycerol kinase (GLPK) mutants in *Escherichia coli*. J Biol Chem 286(26):23150–23159
77. Cheng K-K, Lee B-S, Masuda T, Ito T, Ikeda K, Hirayama A, Deng L, Dong J, Shimizu K, Soga T, Tomita M, Palsson BO, Robert M (2014) Global metabolic network reorganization by adaptive mutations allows fast growth of *Escherichia coli* on glycerol. Nat Commun 5:3233
78. Kornberg HL (2001) Routes for fructose utilization by *Escherichia coli*. J Mol Microbiol Biotechnol 3:355–359
79. Yao R, Kurata H, Shimizu K (2013) Effect of *cra* gene mutation on the metabolism of *Escherichia coli* for a mixture of multiple carbon sources. Adv Biosci Biotechnol 4:477−486
80. Sarkar D, Shimizu K (2008) Effect of *cra* gene knockout together with other genes knockouts on the improvement of substrate consumption rate in *Escherichia coli* under microaerobic conditions. Biochem Eng J 42:224–228
81. Sarkar D, Siddiquee KAZ, Arauzo-Bravo MJ, Oba T, Shimizu K (2008) Effect of *cra* gene knockout together with *edd* and *iclR* genes knockout on the metabolism in *Escherichia coli*. Arch Microbiol 190:559–571
82. Crasnier-Mednansky M, Park MC, Studley WK, Saier MH Jr (1997) Cra-mediated regulations of *Escherichia coli* adenylate cyclase. Microbiology 143:785–792
83. Griffith JK, Baker ME, Rouch DA, Page MG, Skurray RA, Paulsen IT, Chater KF, Baldwin SA, Henderson PJ (1992) Membrane transport proteins: implications of sequence comparisons. Curr Opin Cell Biol 4:684–695
84. Sumiya M, Davis EO, Packman LC, McDonald TP, Henderson PJ (1995) Molecular genetics of a receptor protein for D-xylose, encoded by the gene *xylF*, in *Escherichia coli*. Receptors Channels 3:117–128
85. Song S, Park C (1997) Organization and regulation of the D-xylose operons in *Escherichia coli* K-12: XylR acts as a transcriptional activator. J Bacteriol 179:7025–7032

86. Hasona A, Kim Y, Healy FG, Ingram LO, Shanmugam KT (2004) Pyruvate formate lyase and acetate kinase are essential for anaerobic growth of *Escherichia coli* on xylose. J Bacteriol 186:7593–7600
87. Novotny CP, Englesberg E (1966) The L-arabinose permease system in *Escherichia coli* B/r. Biochim Biophys Acta 117:217–230
88. Schleif R (1969) An L-arabinose binding protein and arabinose permeation in *Escherichia coli*. J Mol Biol 46:185–196
89. Doyle ME, Brown C, Hogg RW, Helling RB (1972) Induction of the *ara* operon of *Escherichia coli* B-r. J Bacteriol 110:56–65
90. Davidson CJ, Narang A, Surette MG (2010) Integration of transcriptional inputs at promoters of the arabinose catabolic pathway. BMC Syst Biol 4:75
91. Madar D, Dekel E, Bren A, Alon U (2011) Negative auto-regulation increases the input dynamical-range of the arabinose system of *Escherichia coli*. BMC Syst Biol 5:111
92. Desai TA, Rao CV (2010) Regulation of arabinose and xylose metabolism in *Escherichia coli*. Appl Environ Microbiol 76:1524–1532
93. Groff D, Benke PI, Batth TS, Bokinsky G, Petzold CJ, Adams PD, Keasling JD (2012) Supplementation of intracellular XylR leads to co-utilization of hemicellulose sugars. Appl Environ Microbiol 78:2221–2229

Transition of the Metabolism during Batch Culture

94. Toya Y, Ishii N, Nakahigashi K, Tomita M, Shimizu K (2010) ^{13}C-metabolic flux analysis for batch culture of *Escherichia coli* and its Pyk and Pgi gene knockout mutants based on mass isotopomer distribution of intracellular metabolites. Biotechnol Prog 26:975–992
95. Schuetz R, Zamboni N, Zampieri M, Heinemann M et al (2012) Multidimensional optimality of microbial metabolism. Science 336:601–604
96. Enjalbert B, Letisse F, Portais J-C (2013) Physiological and molecular timing of the glucose to acetate transition in *Escherichia coli*. Metabolites 3:820–837
97. Xu Y-F, Amador-Noguez D, Reaves ML, Feng XJ et al (2012) Ultrasensitive regulation of anapleurosis via allosteric activation of PEP carboxylase. Nat Chem Biol 8:562–568
98. Ali Azam T, Iwata A, Nishimura A, Ueda S, Ishihama A (1999) Growth phase-dependent variation in protein composition of the *Escherichia coli* nucleoid. J Bacteriol 181:6361–6370
99. Bradley MD, Beach MB, Jason de Koning AP, Pratt TS, Osuna R (2007) Effects of Fis on *Escherichia coli* gene expression during different growth stages. Microbiology 153:2922–2940
100. Mallik P, Pratt TS, Beach MB, Bradley MD, Undamatla J, Osuna R (2004) Growth phase-dependent regulation and stringent control of fis are conserved processes in enteric bacteria and involve a single promoter (fis P) in *Escherichia coli*. J Bacteriol 186:122–135
101. Mallik P, Paul BJ, Rutherford ST, Gourse RL, Osuna R (2006) DksA is required for growth phase-dependent regulation, growth rate-dependent control, and stringent control of fis expression in *Escherichia coli*. J Bacteriol 188:5775–5782
102. Paul BJ, Berkmen MB, Gourse RL (2005) DksA potentiates direct activation of amino acid promoters by ppGpp. PNAS USA 102:7823–7828
103. Ferenci T (2001) Hungry bacteria—definition and properties of a nutritional state. Environ Microbiol 3:605–611
104. Braeken K, Moris M, Daniels R, Vanderleyden J, Michiels J (2006) New horizons for (p) ppGpp in bacterial and plant physiology. Trends Microbiol 14(1):45–54
105. Artsimovitch I, Patlan V, Sekine S, Vassylyeva MN, Hosaka T, Ochi K, Yokoyama S (2004) Structural basis for transcription regulation by alarmone ppGpp. Cell 117:299–310
106. Kanjee U, Ogata K, Houry WA (2012) Direct binding targets of the stringent response alarmone (p)ppGpp. Mol Micobiol 85(6):1029–1043

107. Hengge-Aronis R (2002) Signal transduction and regulatory mechanisms involved in control of the σ^S (RpoS) subunit of RNA polymerase. Microbiol Mol Biol Rev 66:373–395

108. Patten CL, Kirchhof MG, Schertzberg MR, Morton RA, Schellhorn HE (2004) Microarray analysis of RpoS-mediated gene expression in *Escherichia coli* K-12. Mol Genet Genom 272 (5):580–591

109. Jishage M, Kvint K, Shingler V, Nystrom T (2002) Regulation of σ factor competition by the alarmone ppGpp. Genes Dev 16:1260–1270

110. Lacour S, Landini P (2004) σ^S-dependent gene expression at the onset of stationary phase in *Escherichia coli*: function of σ^s-dependent genes and identification of their promoter sequences. J of Bacteriol 186(21):7186–7195

111. Vijayakumar SRV, Kirchhof MG, Patten CL, Schellhorn HE (2004) RpoS-regulated genes of *Escherichia coli* identified by random *lac*Z fusion mutagenesis. J of Bacteriol 186(24):8499–8507

112. Rahman M, Shimizu K (2008) Altered acetate metabolism and biomass production in several *Escherichia coli* mutants lacking *rpoS*-dependent metabolic pathway genes. Mol BioSyst 4 (2):160–169

113. Rahman M, Hasan MM, Shimizu K (2008) Growth phase-dependent changes in the expression of global regulatory genes and associated metabolic pathways in *Escherichia coli*. Biotechnol Lett 30:853–860

114. Llorens NJM, Tormo A, Martínez-García E (2010) Stationary phase in gram-negative bacteria. FEMS Microbiol Rev 34:476–495

115. Desnues B, Cuny C, Gregori G, Dukan S, Aguilaniu H, Nyström T (2003) Differential oxidative damage and expression of stress defence regulons in culturable and non-culturable *Escherichia coli* cells. EMBO Rep 4:400–404

116. Nagamitsu H, Murata M, Kosaka T, Kawaguchi J, Mori H, Yamada M (2013) Crucial roles of MicA and RybB as vital factors for σ^E-Dependent cell lysis in *Escherichia coli* long-term stationary phase. J Mol Microbiol Biotechnol 23:227–232

117. Murata M, Noor R, Nagamitsu H, Tanaka S, Yamada M (2012) Novel pathway directed by sigma E to cause cell lysis in *Escherichia coli*. Genes Cells 17:234–247

118. Noor R, Murata M, Yamada M (2009) Oxidative stress as a trigger for growth phase-specific sigma E-dependent cell lysis in *Escherichia coli*. J Mol Microbiol Biotechnol 17:177–187

Carbon Storage Regulation

119. Yamamotoya T, Dose H, Tian Z, Faure A, Toya Y, Honma M, Igarashi K, Nakahigashi K, Soga T, Mori H, Matsuno H (2012) Glycogen is the primary source of glucose during the lag phase of *E. coli* proliferation. Biochim Biophys Acta 1824:1442–1448

120. Timmermans J, van Melderen L (2010) Post-transcriptional global regulation by CsrA in bacteria. Cell Mol Life Sci 67(17):2897–2908

121. Jonas K, Edwards AN, Ahmad I, Romeo T, Romling U, Melefors O (2010) Complex regulatory network encompassing the Csr, c-di-GMP and motility systems of *Salmonella typhimurium*. Environ Microbiol 12(2):524–540

122. Yakhnin H, Pandit P, Petty TJ, Baker CS, Romeo T, Babitzke P (2007) CsrA of *Bacillus subtilis* regulates translation initiation of the gene encoding the flagellin protein (hag) by blocking ribosome binding. Mol Microbiol 64(6):1605–1620

123. Romeo T, Vakulskas CA, Babitzke P (2013) Post-transcriptional regulation on a global scale: from and function of Csr/Rsm systems. Environ Microbiol 15(2):313–324

124. Dubey AK, Baker CS, Romeo T, Babitzke P (2005) RNA sequence and secondary structure participate in high-affinity CsrA-RNA interaction. RNA 11(10):1579–1587

125. Suzuki K, Wang X, Weilbacher T et al (2002) Regulatory circuitry of the CsrA/CsrB and BarA/UvrY systems of *Escherichia coli*. J of Bacteriol 184(18):5130–5140

126. Weilbacher T, Suzuki K, Dubey AK et al (2003) A novel sRNA component of the carbon storage regulatory system of *Escherichia coli*. Mol Microbiol 48(3):657–670

127. Baker CS, Morozov I, Suzuki K, Romeo T, Babitzke P (2002) CsrA regulates glycogen biosynthesis by preventing translation of *glgC* in *Escherichia coli*. Mol Microbiol 44 (6):1599–1610

128. Dong T, Schellhorn HE (2009) Control of RpoS in global gene expression of *Escherichia coli* in minimal media. Mol Genet Genomics 281:19–33

129. Edwards AN, Patterson-Fortin LM, Vakulskas CA, Mercante JW, Potrykus K, Vinella D, Camacho MI, Fields JA, Thompson SA, Georgellis D et al (2011) Circuitry linking the Csr and stringent response global regulatory systems. Mol Microbiol 80:1561–1580

130. McKee AE, Rutherford BJ, Chivian DC, Baidoo EK, Juminaga D, Kuo D, Benke PI, Dietrich JA, Ma SM, Arkin AP, Petzold CJ, Adams PD, Keasling JD, Chhabra SR (2012) Manipulation of the carbon storage regulator system for metabolite remodeling and biofuel production in *Escherichia coli*. Microb Cell Fact 11:79

131. Revelles O, Millard P, Nougayrede J-P, Dpbrindt U, Osward E, Letisse F, Portais, J-C (2013) The carbon storage regulator (Csr) system exerts a nutrient-specific control over central metabolism in *Escherichia coli* strain Nissle 1917. Plos One 8(6):e66386 (1–12)

132. Tatarko M, Romeo T (2001) Disruption of a global regulatory gene to enhance central carbon flux into phenylalanine biosynthesis in *Escherichia coli*. Current Microbiol 43(1):26–32

Nitrogen Regulation

133. Van Heeswijk WC, Westerhoff HV, Boogerd FC (2013) Nitrogen assimilation in *Escherichia coli*: putting molecular data into a systems perspective. Microbiol Mol Biol Rev 77(4):628–695

134. Kim M, Zhang Z, Okano H, Yan D, Groisman A, Hwa T (2012) Need-based activation of ammonium uptake in *Escherichia coli*. Mol Syst Biol 8:616

135. Gruswitz F, O'Connell J, Stroud RM (2007) Inhibitory complex of the transmembrane ammonia channel, AmtB, and the cytosolic regulatory protein, GlnK, at 1.96 A. PNAS USA 104:42–47

136. Radchenko MV, Thornton J, Merrick M (2010) Control of AmtB-GlnK complex formation by intracellular levels of ATP, ADP, and 2-oxoglutarate. J Biol Chem 285:31037–31045

137. Truan D, Huergo LF, Chubatsu LS, Merrick M, Li XD, Winkler FK (2010) A new P(II) protein structure identifies the 2-oxoglutarate binding site. J Mol Biol 400:531–539

138. Yuan J, Doucette CD, Fowler WU, Feng X-J, Piazza M, Rabitz HA, Wingreen NS, Rabinowitz JD (2009) Metabolomics-driven quantitative analysis of ammonia assimilation in *E. coli*. Mol Syst Biol 5:302

139. Ninfa AJ, Jiang P, Atkinson MR, Peliska JA (2000) Integration of antagonistic signals in the regulation of nitrogen assimilation in *Escherichia coli*. Curr Topics Cell Regul 36:31–75

140. Gerosa L, Kochanowski K, Heinemann M, Sauer U (2013) Dissecting specific and global transcriptional regulation of bacterial gene expression. Mol Syst Biol 9:658

141. Kiupakis AK, Reitzer L (2002) ArgR-independent induction and ArgR-dependent superinduction of the *astCADBE* operon in *Escherichia coli*. J Bacteriol 184(11):2940–2950

142. Commichau FM, Forchhammer K, Stulke J (2006) Regulatory links between carbon and nitrogen metabolism. Curr Opin Microbiol 9(2):167–172

143. Mao XJ, Huo YX, Buck M, Kolb A, Wang YP (2007) Interplay between CRP-cAMP and PII-Ntr systems forms novel regulatory network between carbon metabolism and nitrogen assimilation in *Escherichia coli*. Nucleic Acids Res 35(5):1432–1440

144. 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

145. Jiang P, Ninfa AJ (2007) *Escherichia coli* PII signal transduction protein controlling nitrogen assimilation acts as a sensor of adenylate energy charge in vitro. Biochemistry 46(45): 12979–12996
146. Ninfa J, Jiang P (2005) PII signal transduction proteins: sensors of -ketoglutarate that regulate nitrogen metabolism. Curr Opin Microbiol 8(2):168–173
147. Brauer MJ, Yuan J, Bennet BD, Lu W, Kimball E, Botstein D, Rabinowitz JD (2006) Conservation of the metabolomic response to starvation across two divergent microbes. PNAS USA 103:19302–19307
148. Hart Y, Madar D, Yuan J, Bren A, Mayo AE, Rabinowitz JD, Alon U (2011) Robust control of nitrogen assimilation by a bifunctional enzyme in *E. coli*. Moleculae Cell 41:117–127
149. Doucette CD, Schwab DJ, Wingreen NS, Rabinowitz JD (2011) Alpha-ketoglutarate coordinates carbon and nitrogen utilization via enzyme i inhibition. Nat Chem Biol 7: 894–901
150. You C, Okano H, Hui S, Zhang Z, Kim M, Gunderson CW, Wang Y-P, Lenz P, Yan D, Hwa T (2013) Coordination of bacterial proteome with metabolism by cyclic AMP signaling. Nature 500:301–306
151. Powell BS, Court DL, Inada T, Nakamura Y, Michotey V, Cui X et al (1995) Novel proteins of the phosphotransferase system encoded within the *rpoN* operon of *Escherichia coli*. Enzyme IIANtr affects growth on organic nitrogen and the conditional lethality of an erats mutant. J Biol Chem 270:4822–4839
152. Peterkofski A, Wang G, Seok Y-J (2006) Parallel PTS systems. Arch Biochem Biophys 453 (1):101–107
153. Pflüger-Grau K, Görke B (2010) Regulatory roles of the bacterial nitrogen-related phosphotransferase system. Trend Microbiol 18(5):205–214
154. Lee CR, Cho SH, Yoon MJ, Peterkofsky A, Seok YJ (2007) *Escherichia coli* enzyme IIA^{Ntr} regulates the K$^+$ transporter TrkA. PNAS USA 104:4124–4129
155. Lüttmann D, Heermann R, Zimmer B, Hillmann A, Rampp IS, Jung K, Görke B (2009) Stimulation of the potassium sensor KdpD kinase activity by interaction with the phosphotransferase protein IIA^{Ntr} in *Escherichia coli*. Mol Micobiol 72(4):978–994
156. Lee C-R, Cho S-H, Kim H-J, Kim M, Peterkofsky A, Seok Y-J (2010) Potassium mediates *Escherichia coli* enzyme IIA^{Ntr}-dependent regulation of sigma factor selectivity. Mol Microbiol 78(6):1468–1483
157. Lüttmann D, Göpel Y, Görke B (2012) The phosphotransferase protein EIIA^{Ntr} modulates the phosphate starvation response through interaction with histidine kinase PhoR in *Escherichia coli*. Mol Microbiol 86(1):96–110
158. Kim H-J, Lee C-R, Kim M, Peterkofsky A, Seok Y-J (2011) Dephosphorylated NPr of the nitrogen PTS regulates lipid a biosynthesis by direct interaction with LpxD. Biochem Biophys Res Commun 409(3):556–561
159. Lee C-R, Park Y-H, Kim M, Kim Y-R, Park S, Peterkofsky A, Seok Y-J (2013) Reciprocal regulation of the autophosphorylation of enzyme I^{Ntr} by glutamine and α-ketoglutarate in *Escherichia coli*. Mol Microbiol 88(3):473–485

Sulfur Regulation

160. Bykowski T, van der Ploeg JR, Iwanicka-Nowicka R, Hryniewicz MM (2002) The switch from inorganic to organic sulfur assimilation in *Escherichia coli*: adenosine 5′-phosphosulphate (APS) as a signalling molecule for sulphate excess. Mol Microbiol 43:1347–1358
161. Kredich NM (1996) Biosynthesis of cysteine. In: Neidhardt FC, Curtiss R III, Ingraham JL, Lin ECC, Low KB, Magasanik B, Reznikoff WS, Riley M, Schaechter M, Umbarger HH (eds) *Escherichia coli* and *Salmonella*: cellular and molecular biology, vol 1, 2nd edn. ASM Press, Washington DC, pp 514–527

162. Iwanicka-Nowicka R, Hryniewicz MM (1995) A new gene, *cbl*, encoding a member of the LysR family of transcriptional regulators belongs to *Escherichia coli cys* regulon. Gene 166:11–17
163. Zimmer DP, Soupene E, Lee HL, Wendisch VF, Khodursky BJ, Peter R, Bender A, Kustu S (2000) Nitrogen regulatory protein controlled genes of *Escherichia coli*: scavenging as a defense against nitrogen limitation. PNAS USA 97:14674–14679
164. Gyaneshwar P, Paliy O, McAuliffe J, Popham DL, Jordan MI, Kustu S (2005) Sulfur and Nitrogen Limitation in *Escherichia coli* K-12: specific homeostatic responses. J Bacteriol 187 (3):1074–1090

Phosphate Regulation

165. Wanner BL (1996) Phosphorus assimilation and control of the phosphate regulon. In: Neidhardt FC, Curtiss IIIR, Ingraham JL et al (eds) *Escherichia coli* and salmonella: cellular and molecular biology, pp 1357–1381. ASM Press, Washington DC
166. Wanner BL (1993) Gene regulation by phosphate in enteric bacteria. J Cellr Biochem 51 (1):47–54
167. Baek JH, Lee SY (2007) Transcriptome analysis of phosphate starvation response in *Escherichia coli*. J Microbiol Biotechnol 17(2):244–252
168. Van Dien SJ, Keasling JD (1998) A dynamic model of the *Escherichia coli* phosphate-starvation response. J Theoret Biol 190(1):37–49
169. Marzan LW, Shimizu K (2011) Metabolic regulation of *Escherichia coli* and its phoB and phoR gene knockout mutants under phosphate and nitrogen limitations as well as acidic condition. Microb Cell Fact 10:39
170. Spira B, Silberstein N, Yagil E (1995) Guanosine 3′,5′-bispyrophosphate (ppGpp) synthesis in cells of *Escherichia coli* starved for Pi. J Bacteriol 177(14):4053–4058

Metal Ion Regulation and Oxidative Stress Regulation

171. Frey PA, Reed GH (2012) The ubiquity of iron. ACS Chem Biol 7:1477–1481
172. Dixon SJ, Stockwell BR (2014) The role of iron and reactive oxygen species in cell death. Nat Chem Biol 10:9–17
173. Dann CEIII, Wakeman CA, Sieling CL, Baker SC, Irnov I, Winker WC (2007) Structure and mechanism of a metal-sensing regulatory RNA. Cell 130:878–892
174. Helmann JD (2007) Measuring metals with RNA. Mol Cell 27:859–860
175. Zheng M, Doan B, Schneider TD, Storz G (1999) OxyR and SoxRS regulation of fur. J Biotechnol 181:4639–4643
176. McHugh JP, Rodríguez-Quiñones F, Abdul-Tehrani H, Svistunenko DA, Poole RK, Cooper CE, Andrews SC (2003) Global iron-dependent gene regulation in *Escherichia coli*. J Biol Chem 278:29478–29486
177. Storz G, Imlay JA (1999) Oxidative stress. Curr Opin Microbiol 2:188–194
178. Jovanovic G, Lloyde LJ, Stumpf MP, Mayhew AJ, Buck M (2006) Induction and function of the phase shock protein extracytoplasmic stress response in *Escherichia coli*. J Biol Chem 281:21147–21161
179. Blanchard JR, Wholey WY, Conlon EM, Pomposiello PJ (2007) Rapid changes in gene expression dynamics in response to superoxide reveal SoxRS dependent and independent transcriptional network. PLoS One 2:e1186
180. Brynildsen MP, Liao JC (2009) An integrated network approach indentifies the isobutanol response network of *Escherichia coli*. Mol Syst Biol 5:34

181. Braun V, Hantke K, Koster W (1998) Bacterial ion transport: mechanisms, genetics, and regulation. In: Sigel A, Sigel H (eds) Metal ions in biological systems. Marcel Dekker, New York, pp 67–145

182. Azpiroz MF, Laviña M (2004) Involvement of enterobactin synthesis pathway in production of Microcin H47. Antimicrob Agents Chemother 48:1235–1241

183. Semsey S, Andersson AM, Krishna S, Jensen MH, Masse E, Sneppen K (2006) Genetic regulation of fluxes: iron homeostasis of *Escherichia coli*. Nucleic Acids Res 34:4960–4967

184. Hantash FM, Ammerlaan M, Earhart CF (1997) Enterobactin synthase polypeptides of *Escherichia coli* are present in an osmotic-shock-sensitive cytoplasmic locality. Microbiol 143:147–156

185. 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

186. Zhang Z, Gosset G, Barabote R, Gonzalez CS, Cuevas WA, Saier MH Jr (2005) Functional interactions between the carbon and iron utilization regulators, Crp and Fur, in *Escherichia coli*. J Bacteriol 187:980–990

187. Perera IC, Grove A (2010) Molecular mechanisms of ligand-mediated attenuation of DNA binding by MarR family transcriptional regulators. J Mol Cell Biol 2:243–254

188. Hao Z, Lou H, Zhu R, Zhu J, Zhang D, Zhao BS, Zeng S, Chen X, Chan J, He C, Chen PR (2014) The multiple antibiotic resistance regulator MarR is a copper sensor in *Escherichia coli*. Nat Chem Biol 10:21–28

189. Pomposiello PJ, Demple B (2001) Redox-operated genetic switches: the SoxR and OxyR transcription factors. Trends Biotechnol 19(3):109–114

190. Gaudu P, Weiss B (1996) SoxR, a [2Fe–2S] transcription factor, is active only in its oxidized form. PNAS USA 93(19):10094–10098

191. Mailloux RJ, Bériault R, Lemire J, Singh R, Chénier RR, Hamel RD, Appanna VD (2007) The tricarboxylic acid cycle, an ancient metabolic network with a novel twist. Plos One 2(8): e690

192. DeNicola GM et al (2011) Oncogene-induced Nrf2 transcription promotes ROS detoxification and tumorigenesis. Nature 475:106–109

193. Son J et al (2013) Glutamine supports pancreatic cancer growth through a KRAS-regulated metabolic pathway. Nature 496:101–105

194. Maddocks OD et al (2013) Serine starvation induces stress and p53-dependent metabolic remodeling in cancer cells. Nature 493:542–546

195. Trachootham D, Alexandre J, Huang P (2009) Targeting cancer cells by ROS-mediated mechanisms: a radical therapeutic approach ? Nat Rev Drug Discov 8:579–591

Redox State Regulation

196. Gunsalus RP (1992) Control of electron flow in *Escherichia coli*: coordinated transcription of respiratory pathway genes. J Bacteriol 174(22):7069–7074

197. 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(32):29837–29855

198. Alexeeva S, Hellingwerf KJ, de Mattos MJT (2003) Requirement of ArcA for redox regulation in *Escherichia coli* under microaerobic but not anaerobic or aerobic conditions. J Bacteriol 185(1):204–209

199. 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(6):619–627

200. Georgellis D, Kwon O, Lin ECC (2001) Quinones as the redox signal for the arc two-component system of bacteria. Science 292(5525):2314–2316

201. Malpica R, Franco B, Rodriguez C, Kwon O, Georgellis D (2004) Identification of a quinone-sensitive redox switch in the ArcB sensor kinase. PNAS USA 101(36):13318–13323

202. Constantinidou C, Hobman JL, Grifiths L, Patel MD, Penn CW, Cole JA, Overton TW (2006) A reassessment of the FNR regulon and transcriptomic analysis of the effects of nitrate, nitrite, NarXL, and NarQP as *Escherichia coli* K12 adapts from aerobic to anaerobic growth. J Biol Chem 281(8):4802–4815

203. Kessler D, Knappe J (1996) Anaerobic dissimilation of pyruvate. In: Neidhardt FC, Curtiss R, Ingraham JI et al (eds) *E. Coli* and salmonella: cellular and molecular biology, 2nd edn, vol 1, pp 199–205. ASM Press, Washington, DC

204. Toya Y, Nakahigashi K, Tomita M, Shimizu K (2012) Metabolic regulation analysis of wild-type and *arcA* mutant *Escherichia coli* under nitrate conditions using different levels of omics data. Mol Biosyst 8:2593–2604

205. Maeda T, Sanchez-Torres V, Wood TK (2007) Enhanced hydrogen production from glucose by metabolically engineered *Escherichia coli*. Appl Microbiol Biotechnol 77:879–890

206. Zhu J, Shimizu K (200) The effect of *pfl* genes knockout on the metabolism for optically pure d-lactate production by *Escherichia coli*. Applied Microbiol Biotechnol 64:367–75

207. Zhu J, Shimizu K (2005) Effect of a single-gene knockout on the metabolic regulation in *E. coli* for d-lactate production under microaerobic conditions. Metabolic Eng 7:104–115

208. Vemuri GN, Altman E, Sangurdekar DP, Khodursky AB, Eiteman MA (2006) Overflow metabolism in *Escherichia coli* during steady-state growth: transcriptional regulation and effect of the redox ratio. Appl Environ Microbiol 72(5):3653–3661

209. Valgepea K, Adamberg K, Seiman A, Vilu R (2014) Escherichia coli achieves faster growth by increasing catalytic and translation rates of proteins. Mol BioSyst 9:2344–2358

210. Nizam SA, Zhu J, Ho PY, Shimizu K (2009) Effects of *arcA* and *arcB* genes knockout on the metabolism in *Escherichia coli* under aerobic condition. Biochem Eng J 44(2–3):240–250

211. Vemuri GN, Eiteman MA, Altman E (2006) Increased recombinant protein production in *Escherichia coli* strains with overexpressed water-forming NADH oxidase and a deleted ArcA regulatory protein. Biotechnol Bioeng 94(3):538–542

212. Nizam SA, Shimizu K (2008) Effects of *arc* A and *arc* B genes knockout on the metabolism in *Escherichia coli* under anaerobic and microaerobic conditions. Biocheml Eng J 42:229–236

Acid Shock

213. Foster JW (2004) Escherichia coli acid resistance: tales of an amateur acidophile. Nat Rev Microbiol 2(11):898–907

214. Stincone A, Daudi N, Rahman AS et al (2011) A systems biology approach sheds new light on *Escherichia coli* acid resistance. Nucleic Acids Res 39(17):7512–7528

215. Richard HT, Foster JW (2003) Acid resistance in *Escherichia coli*. Adv Appl Microbiol 52:167–186

216. Richard HT, Foster JW (2004) *Escherichia coli* glutamate-and arginine-dependent acid resistance systems increase internal pH and reverse transmembrane potential. J Bacteriol 86 (18):6032–6041

217. Gong S, Richard H, Foster JW (2003) YjdE (AdiC) is the arginine: agmatine antiporter essential for arginine-dependent acid resistance in *Escherichia coli*. J Bacteriol 185 (15):4402–4409

218. Iyer R, Williams C, Miller C (2003) Arginine-agmatine antiporter in extreme acid resistance in *Escherichia coli*. J Bacteriol 185:6556–6561

219. Martin-Galiano AJ, Ferrandiz MJ, de La Campa AG (2001) The promoter of the operon encoding the F0F1 ATPase of *Streptococcus pneumonia* is inducible by pH. Molr Microbiol 41:327–338

220. Castanie-Cornet MP, Foster JW (2001) *Escherichia coli* acid resistance: cAMP receptor protein and a 20 bp *cis*-acting sequence control pH and stationary phase expression of the *gadA* and *gadBC* glutamate decarboxylase genes. Microbiol 147:709–715

221. Marzan LW, Shimizu K (2011) Metabolic regulation of *Escherichia coli* and its *phoB* and *phoR* genes knockout mutants under phosphate and nitrogen limitations as well as at acidic condition. Microb Cell Fact 10:39

222. Greenberg JT, Monach P, Chou JH, Josephy PD, Demple B (1990) Positive control of a global antioxidant defense regulon activated by superoxide-generating agents in *Escherichia coli*. PNAS USA 87(16):6181–6185

223. Suziedeliene E, Suziedelis K, Garbenciute V, Normark S (1999) The acid-inducible asr gene in *Escherichia coli*: transcriptional control by the phoBR operon. J Bacteriol 181(7):2084–2093

224. Wu X, Altman R, Eiteman MA, Altman E (2013) Effect of overexpressing *nhaA* and *nhaR* on sodium tolerance and lactate production in *Escherichia coli*. J Biol Eng 7:3

Heat Shock

225. Kitagawa M, Miyakawa M, Matsumura Y, Tsuchido T (2002) *Escherichia coli* small heat shock proteins, IbpA and IbpB, protect enzymes from inactivation by heat and oxidants. Eur J Biochem 269(12):2907–2917

226. Sørensen HP, Mortensen KK (2005) Soluble expression of recombinant proteins in the cytoplasm of *Escherichia coli*. Microb Cell Fact 4:1

227. Hoffmann F, Weber J, Rinas U (2002 Metabolic adaptation of *Escherichia coli* during temperature-induced recombinant protein production: 1. Readjustment of metabolic enzyme synthesis. Biotechnol Bioeng 80(3):313–319

228. Tilly K, Erickson J, Sharma S, Georgopoulos C (1986) Heat shock regulatory gene *rpoH* mRNA level increases after heat shock in *Escherichia coli*. J Bacteriol 168(3):1155–1158

229. Tilly K, Spence J, Georgopoulos C (1989) Modulation of stability of the *Escherichia coli* heat shock regulatory factor ^{32}. J Bacteriol 171(3):1585–1589

230. Shin D, Lim S, Seok YJ, Ryu S (2001) Heat shock RNA polymerase (E ^{32}) is involved in the transcription of *mlc* and Crucial for Induction of the Mlc regulon by glucose in *Escherichia coli*. J Biol Chem 276(28):25871–25875

231. Hasan CM, Shimizu K (2008) Effect of temperature up-shift on fermentation and metabolic characteristics in view of gene expressions in *Escherichia coli*. Microb Cell Fact 7:35

232. Kumari S, Beatty CM, Browning DF et al (2000) Regulation of acetyl coenzyme A synthetase in *Escherichia coli*. J Bacteriol 182(15):4173–4179

233. Browning DF, Beatty CM, Wolfe AJ, Cole JA, Busby SJW (2002) Independent regulation of the divergent *Escherichia coli nrfA* and *acs*P1 promoters by a nucleoprotein assembly at a shared regulatory region. Mol Microbiol 43(3):687–701

234. Beatty CM, Browning DF, Busby SJW, Wolfe AJ (2003) Cyclic AMP receptor protein-dependent activation of the *Escherichia coliacs* P2 promoter by a synergistic class III mechanism. J Bacteriol 185(17):5148–5157

235. Browning DF, Beatty CM, Sanstad EA, Gunn KE, Busby SJW, Wolfe AJ (2004) Modulation of CRP-dependent transcription at the *Escherichia coli* acsP2 promoter by nucleoprotein complexes: anti-activation by the nucleoid proteins FIS and IHF. Mol Microbiol 51(1):241–254

236. Privalle CT, Fridovich I (1987) Induction of superoxide dismutase in *Escherichia coli* by heat shock. PNAS USA 84(9):2723–2726

Cold Shock

237. Etchegaray J-P, Inoue M (1999) CspA, CspB, and CspG, major cold shock proteins of *Escherichia coli*, are induced at low temperature under conditions that completely block protein synthesis. J Bacteriol 181(6):1827–1830
238. White-Ziegler CA, Um S, Perez NM, Berns AL, Malhowski AJ, Young S (2008) Low temperature (23 °C) increases expression of biofilm-, cold shock-, and RpoS-dependent genes in *Escherichia coli* K-12. Microbiol 154:148–166
239. Kim Y-H, Han KY, Lee K, Lee J (2005) Proteome response of *Escherichia coli* fed-batch culture to temperature downshift. Appl Microbiol Biotechnol 68:786–793

Solvent Stress Regulation

240. Dunlop MJ (2011) Engineering microbes for tolerance to next-generation biofuels. Biotechnol Biofuels 4:32
241. Isken S, de Bont JAM (1998) Bacteria tolerant to organic solvents. Extremophiles 2:229–238
242. Ramos JL, Duque E, Gallegos M-T, Godoy P, Ramos-Gonzalez MI, Rojas A, Teran W, Segura A (2002) Mechanisms of solvent tolerance in Gram-negative bacteria. Annu Rev Microbiol 56:743–768
243. Nicolaou SA, Gaida SM, Papoutsakis ET (2010) A comparative view of metabolite and substrate stress and tolerance in microbial bioprocessing: from biofuels and chemicals, to biocatalysis and bioremediation. Metab Eng 12:307–331
244. Takatsuka Y, Chen C, Nikaido H (2010) Mechanism of recognition of compounds of diverse structures by the multidrug efflux pump AcrB of *Escherichia coli*. PNAS USA 107:6559–6565
245. Ankarloo J, Wikman S, Nicholls IA (2010) *Escherichia coli mar* and *acrAB* mutants display no tolerance to simple alcohols. Int J Mol Sci 11:1403–1412
246. Piper P (1995) The heat-shock and ethanol stress responses of yeast exhibit extensive similarity and functional overlap. FEMS Microbiol Lett 134:121–127
247. Rutherford BJ, Dahl RH, Price RE, Szmidt HL, Benke PI, Mukhopadhyay A, Keasling JD (2010) Functional genomic study of exogenous n-butanol stress in *Escherichia coli*. Appl Environ Microbiol 76:1935–1945
248. Tomas C, Beamish J, Papoutsakis E (2004) Transcriptional analysis of butanol stress and tolerance in *Clostridium acetobutylicum*. J Bacteriol 186:2006–2018
249. Fiocco D, Capozzi V, Goffin P, Hols P, Spano G (2007) Improved adaptation to heat, cold, and solvent tolerance in *Lactobacillus plantarum*. Appl Microbiol Biotechnol 77:909–915
250. Reyes LH, Almario MP, Kao KC (2011) Genomic library screens for genes involved in n-butanol tolerance in *Escherichia coli*. PLoS One 6:e17678
251. Sikkema J, de Bont JAM, Poolman B (1995) Mechanisms of membrane toxicity of hydrocarbons. Microbiol Rev 59:201–222
252. Holtwick R, Meinhardt F, Keweloh H (1997) cis-trans isomerization of unsaturated fatty acids: cloning and sequencing of the cti gene from Pseudomonas putida P8. Appl Environ Microb 63:4292–4297
253. Kiran M, Prakash J, Annapoorni S, Dube S, Kusano T, Okuyama H, Murata N, Shivaji S (2004) Psychrophilic Pseudomonas syringae requires transmonounsaturated fatty acid for growth at higher temperature. Extremophiles 8:401–410

Osmoregulation

254. Kramer R (2010) Bacterial stimulus perception and signal transduction: response to osmotic stress. Chem Res 10:217–229
255. Wood JM (2011) Bacterial osmoregulation: a paradigm for the study of cellular homeostasis. Ann Rev Microbiol 65:215–238
256. Walderhaug MO, Polarek JW, Voelkner P, Daniel JM, Hesse JE, Altendorf K, Epstein W (1992) KdpD and KdpE, proteins that control expression of the *kdpABC* operon, are members of the two-component sensor-effector class of regulators. J Bacteriol 174:2152–2159
257. Sugiura A, Hirokawa K, Nakashima K, Mizuno T (1994) Signal-sensing mechanisms of the putative osmosensor KdpD in *Escherichia coli*. Mol Microbiol 14:929–938
258. Hamann K, Zimmann P, Altendorf K (2008) Reduction of turgor is not the stimulus for the sensor kinase KdpD of *Escherichia coli*. J Bacteriol 190:2360–2367
259. Heermann R, Jung K (2010) The complexity of the 'simple' two-component system KdpD/KdpE in *Escherichia coli*. Microbiol Lett 304:97–106
260. Sevin DC, Sauer U (2014) Ubiquinone accumulation improves osmotic-stress tolerance in *Escherichia coli*. Nat Chem Biol 10:266–272
261. Piuri M, Sanchez-Rivas C, Ruzal SM (2005) Cell wall modifications during osmotic stress in *Lactobacullus casei*. J Appl Microbiol 98:84–95
262. Clarke CF, Rowat AC, Gober JW (2014) Is CoQ a membrane stabilizer? Nat Chem Biol 10:242–243
263. Calamita G, Bishai WR, Preston GM, Guggino WB, Agre P (1995) Molecular cloning and characterization of AqpZ, a water channel from *Escherichia coli*. J Biol Chem 270:29063–29066
264. Lamins LA, Rhoads DB, Epstein W (1981) Osmotic control of kdp operon expression in *Escherichia coli*. PNAS USA 78:464–468
265. Record MT Jr, Courtenary ES, Cayley DS, Guttman HJ (1998) Responses of *E. coli* to osmotic stress: large changes in amounts of cytoplasmic solutes and water. Trends Biochem Sci 23:143–148
266. Grothe S, Krogsrud RL, McClellan DJ, Milner JL, Wood JM (1986) Proline transport and osmotic response in *Escherichia coli* K-12. J Bacteriol 166:253–259
267. Von Weyman N, Nyyssola A, Reinikainen T, Leisola M, Ojamo H (2001) Improved osmotolerance of recombinant *Escherichia coli* by de novo glycine betaine biosynthesis. Appl Microbiol Biotechnol 55:214–218
268. Giaever HM, Styrvoid OB, Kaasen I, Strom AR (1998) Biochemical and genetic characterization of osmoregulatory trehalose synthesis in *Escherichia coli*. J Bacteriol 170:2841–2849
269. Elbein AD, Pan YT, Pastuszak I, Carroll D (2003) New insights on trehalose: a multifunctional molecule. Glycobiology 13:17R–27R
270. Klein W, Ehmann U, Boos W (2991) The repression of trehalose transport and metabolism in *Escherichia coli* by high osmolarity is mediated by trehalose-6-phosphate phosphatase. Res Microbiol 142:359–371
271. Turunen M, Olsson J, Dallner G (2004) Metabolism and function of coenzyme Q. Biochim Biophys ACTA Biomembr 1660:171–199

Biofilm, Motility and Quarum Sensing

272. Wang X, Dubey AK, Suzuki K, Baker CS, Babitzke P, Romeo T (2005) CsrA post-transcriptionally represses pgaABCD, responsible for synthesis of a biofilm polysaccharide adhesin of *Escherichia coli*. Mol Microbiol 56:1648–1663

273. Hengge R (2009) Principles of c-di-GMP signalling in bacteria. Nat Rev Microbiol 7:263–273
274. Barnhart MM, Chapman MR (2006) Curli biogenesis and function. Annu Rev Microbiol 60:131–147
275. Thomason MK, Fontaine F, De Lay N, Storz1 G (2012) A small RNA that regulates motility and biofilm formation in response to changes in nutrient availability in *Escherichia coli*. Mol Microbiol 84(1):17–35
276. Jackson DW, Simecka JW, Romeo T (2002) Catabolite repression of *Escherichia coli* biofilm formation. J Bacteriol 184:3406–3410
277. Waters CM, Bassler BL (2005) Quorum sensing: cell-to-cell communication in bacteria. Annu Rev Cell Dev Biol 21:319–346
278. De Lay N, Gottesman S (2009) The Crp-activated small noncoding regulatory RNA CyaR (RyeE) links nutritional status to group behavior. J Bacteriol 191:461–476

Systems Biology Approach

279. Yu Matsuoka, Shimizu K (2015) Current status and future perspectives of kinetic modeling for the cell metabolism with incorporation of the metabolic regulation mechanism. Bioreses Bioprocess 2:4
280. Almquist J, Cvijovic M, Hatzimanikatis V, Nielsen J, Jirstrand M (2014) Kinetic models in industrial biotechnology-improving cell factory performance. Metab Eng 24:38–60
281. Chassagnole C, Noisommitt-Rizzi N, Schmid JW, Mauch K, Reuss M (2002) Dynamic modeling of the central carbon metabolism of *Escherichia coli*. Biotechnol Bioeng 79:53–73
282. Kadir TA, 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 9:88
283. Nishio Y, Usuda Y, Matsui K, Kurata H (2008) Computer-aided rational design of the phosphotransferase system for enhanced glucose uptake in *Escherichia coli*. Mol Syst Biol 4:160
284. Usuda Y, Nishio Y, Iwatani S, Van Dien SJ, Imaizumi A, Shimbo K, Kageyama N, Iwahata D, Miyano H, Matsui K (2010) Dynamic modeling of *Escherichia coli* metabolic and regulatory systems for amino-acid production. J Biotechnol 147:17–30
285. Matsuoka Y, Shimizu K (2013) Catabolite regulation analysis of *Escherichia coli* for acetate overflow mechanism and co-consumption of multiple sugars based on systems biology approach using computer simulation. J Biotechnol 168:155−173

Concluding Remarks

286. Gutierrez-Rios RM, Rosenblueth DA, Loza JA, Huerta AM, Glasner JD, Blattner FR, Collado-Vides J (2003) Regulatory network of *Escherichia coli*: consistency between literature knowledge and microarray profiles. Genome Res 13:2435–2443
287. Maheswaran M, Forchhammer K (2003) Carbon-source-dependent nitrogen regulation in *Escherichia coli* is mediated through glutamine-dependent GlnB signaling. Microbiology 149:2163–2172
288. Quan JA, Schneider BL, Paulsen IT, Yamada M, Kredich NM, Saier MH Jr (2002) Regulation of carbon utilization by sulfur availability in *Escherichia coli* and *Salmonella typhimurium*. Microbiology 148:123–131
289. De Lorenzo V, Herrero M, Giovannini F, Neilands JB (1998) Fur (ferric uptake regulation) protein and CAP (catabolite-activator protein) modulate transcription of *fur* gene in *Escherichia coli*. Eur J Biochem 173:537–546

290. Zhang Z, Gosset G, Barabote R, Gonzalez CS, Cuevas WA, Saier MH Jr (2005) Functional interactions between the carbon and iron utilization regulators, Crp and Fur, *Escherichia coli*. J Bacteriol 187(3):980–990
291. Gyaneshwar P, Paliy O, McAuliffe J, Popham DL, Jordan MI, Kustu S (2005) Sulfur and nitrogen limitation in *Escherichia coli* K-12: specific homeostatic responses. J Bacteriol 187 (3):1074–1090
292. Dragosits M, Mozhayskiy V, Quinones-Soto S, Park J, Tagkopoulos I (2013) Evolutionary potential, cross-stress behavior and the genetic basis of acquired stress resistance in *Escherichia coli*. Mol Syst Biol 9:643
293. Weber H, Polen T, Heuveling J, Wendisch V, Hengge R (2005) Genome-wide analysis of the general stress response network in *Escherichia coli*: sigmaS-dependent genes, promoters, and sigma factor selectivity. J Bacteriol 187:1591–1603
294. Jenkins D, Schultz J, Matin A (1988) Starvation-induced cross protection against heat or H_2O_2 challenge in *Escherichia coli*. J Bacteriol 170:3910–3914
295. Jenkins D, Auger E, Matin A (1991) Role of RpoH, a heat shock regulator protein, in *Escherichia coli* carbon starvation protein synthesis and survival. J Bacteriol 173:1992–1996
296. Gunasekera T, Csonka L, Paliy O (2008) Genome-wide transcriptional responses of *Escherichia coli* K-12 to continuous osmotic and heat stresses. J Bacteriol 190:3712–3720
297. White-Ziegler C, Um S, Pérez N, Berns A, Malhowski A, Young S (2008) Low temperature (23 degrees C) increases expression of biofilm-, cold-shock- and RpoS-dependent genes in *Escherichia coli* K-12. Microbiology 154:148–166
298. Rutherford B, Dahl R, Price R, Szmidt H, Benke P, Mukhopadhyay A, Keasling J (2012) Functional genomic study of exogenous n-butanol stress in *Escherichia coli*. Appl Environ Microbiol 76:1935–1945
299. Rabinowitz J, Silhavy TJ (2012) Metabolite turns master regulator. Nature 500:283–284
300. Klumpp S, Zhang Z, Hwa T (2009) Growth rate-dependent global effects on gene expression in bacteria. Cell 139:1366–1375
301. Scott M, Gunderson CW, Mateescu EM, Zhang Z, Hwa T (2010) Interdependence of cell growth and gene expression: origins and consequences. Science 330:1099–1102
302. Koebman BJ, Westerhoff HV, Snoep JL, Nilsson D, Jensen PR (2002) The glycolytic flux in *Escherichia coli* is controlled by the demand for ATP. J Bacteriol 184:3909
303. Luo Y, Zhang T, Wu H (2014) The transport and mediation mechanisms of the common sugars in *Escherichia coli*. Biotechnol Adv 32:905–919
304. Yao R, Shimizu K (2012) Recent progress in metabolic engineering for the production of biofuels and biochemical from renewable sources with particular emphasis on catabolite regulation and its modulation. Process Biochem 48(9):1409–1417
305. Aidelberg G, Towbin BD, Rothschild D, Dekel E, Bren A, Alon U (2014) Hierarchy of non-glucose sugars in *Escherichia coli*. BMC Syst Biol 8:133
306. Balsalobre C, Johansson J, Uhlin BE (2006) Cyclic AMP-dependent osmoregulation of crp gene expression in *Escherichia coli*. J Bacteriol 188(16):5935–5944
307. Zhang H, Chong H, Ching CB, Jiang R (2012) Random mutagenesis of global transcription factor cAMP receptor protein for improved osmotolerance. Biotechnol Bioeng 109(5):1165–1172
308. Basak S, Jiang R (2012) Enhancing E. coli tolerance towards oxidative stress via engineering its global regulator cAMP receptor protein (CRP). PLOS One 7(12):e51179
309. Basak S, Geng H, Jiang R (2013) Rewiring global regulator cAMP receptor protein (CRP) to improve E. coli tolerance towards low pH. J Biotechnol 173:68–75
310. Chong H, Yeow J, Wang I, Song H, Jiang R (2013) Improving acetate tolerance of *Escherichia coli* by rewiring its global regulator cAMP receptor protein (CRP). PLOS One 8 (10):e77422
311. Chong H, Huang L, Yeow J, Wang I, Zhang H, Song H, Jiang R (2013) Improving ethanol tolerance of Escherichia coli by rewiring its global regulator cAMP receptor protein (CRP). PLOS One 8(2):e57628

312. Zhang H, Chong H, Ching CB, Song H, Jiang R (2012) Engineering global transcription factor cyclic AMP receptor protein of *Escherichia coli* for improved 1-butanol tolerance. Appl Microbiol Biotechnol 94:1107–1117
313. Khankal R, Chin JW, Ghosh D, Cirino PC (2009) Transcriptional effects of CRP* expression in *Escherichia coli*. J Biol Eng 3:13
314. Gosset G, Zhang Z, Nayyar S, Cuevas WA, Saier MH Jr (2004) Transcriptome analysis of Crp-dependent catabolite control of gene expression in *Escherichia coli*. J Bacteriol 186 (11):3516–3524
315. Valgepea K, Adamberg K, Seiman A, Vilu R (2014) *Escherichia coli* achieves faster growth by increasing catalytic and translation rates of proteins. Mol BioSyst 9:2344–2358
316. Baste DJV, Nol K, Niedenfuhr Mendum TA, Hawkins ND, Ward JL, Beale MH, Wiechert W, McFadden J (2013) ^{13}C-Flux spectral analysis of host-pathogen metabolism reveals a mixed diet for intracellular *Mycobacterium tuberculosis*. Chem Biol 20:1–10
317. Shimizu K (2004) Metabolic flux analysis based on ^{13}C-labeling experiments and integration of the information with gene and protein expression patterns. Adv Biochem Eng Biotechnol 91:1–49
318. Shimizu K (2013) Bacterial cellular metabolic systems. Woodhead Publ. Co., Oxford