2

Brief overview of metabolic regulation of a bacterial cell

Abstract: Metabolic regulation of main metabolism is explained based on protein expressions for the batch culture of *Escherichia coli*. The metabolic regulation mechanism is briefly explained for aerobic and anaerobic cultivations of *E. coli* using glucose as a carbon source. Moreover, the metabolic regulations using gluconate, glycerol, or acetate as a carbon source are explained. The metabolic changes during batch cultivation of *E. coli* are also described at the exponential growth phase, early stationary phase, and stationary phase, based on gene expression data.

Key words: metabolic regulation of *E. coli*; proteome; aerobic and anaerobic cultivations; gluconate metabolism; glycerol metabolism; acetate metabolism; stationary phase; RpoS.

2.1 Introduction

All organisms respond to environmental variations, such as nutrient availability, oxygen limitation, etc. Corresponding physiological changes are accompanied by gene and protein expressions, etc. A better understanding of gene and protein expressions for cells under physiological changes is of primary importance. The most important notion is to regard the cell as a whole in its entirety in relation to global metabolic regulation, rather than through individual reactions (Shimizu, 2004; 2009). In this chapter, metabolic regulation of a bacterial cell such as *E. coli*, in response to different carbon sources and lower oxygen concentrations, is explained based on protein expressions, etc. Then we

consider how metabolism changes with respect to time during the batch culture, and finally, metabolisms for acetate formation in *E. coli* are described.

2.2 Metabolic regulation analysis by protein expressions

As early as 1975, 2D electrophoresis (2DE) was proposed as the most efficient method of separating complex protein mixtures to analyze global patterns of gene expressions at the protein level (O'Farrell, 1975). Since then, this approach has been widely adopted (Anderson and Anderson, 1998; Gygi et al., 2000a,b; Han and Lee, 2006). One major outcome of these proteome studies is the establishment of 2DE databases for many organisms. These databases provide easy access to the analysis of gene expression in response to genetic or environmental alterations at the protein level. Usually, we choose a condition of interest, and study the global protein responses of the organism to the designated condition, both qualitatively (translational or post-translational modifications) and quantitatively (relative abundance or coordinated expression). In fact, the regulation of metabolic processes ultimately depends upon the control of enzyme activity.

There are three general mechanisms by which the activity of enzymes can be regulated: control by reversible binding of effectors; by covalent modification; and by alteration of enzyme concentration. In the first case, the enzyme is activated or inhibited by binding of a signal molecule, which may or may not be the substrate or product of the enzyme reaction, to the specific regulatory site, producing a conformational change. Substrate effect and allosteric control may be the example. In the case of covalent modification, the structure of the enzyme can be altered by the action of other enzymes. There are a number of such examples, such as the regulation by phosphorylation, i.e. phosphate is incorporated into the enzyme by a protein kinase using ATP, and is removed by a protein phosphatase. The third mechanism that regulates the enzyme activity is the alteration of the concentration of enzyme protein in the cell. The concentration of a protein in the cell is governed by the rate of the synthesis and the rate of degradation. The rate of synthesis of a particular protein may be controlled at several different levels. The rate of transcription of the gene may also be controlled. Other possible sites of control are the processing of the transcript to give mRNA, the

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transfer of mRNA out of the nucleus, the rate of degradation of mRNA in the cytoplasm, or the rate of translation of mRNA to form the protein on the ribosome. There is strong evidence that the rate of transcription is under rigorous control, and this control is important in determining the enzyme profile of a particular cell type (Martin, 1987). Therefore, to a large extent, enzyme activity reflects the protein expression level.

2.2.1 Fermentation characteristics and protein expressions with enzyme activities

Figure 2.1 shows the time courses of the batch cultivations for different carbon sources and under anaerobic conditions (Peng and Shimizu, 2003). As shown in Figure 2.1, *E. coli* grown in glucose or gluconate medium excreted acetate aerobically. Upon exhaustion of glucose or gluconate, the cells synthesized the enzymes of glyoxylate by-pass, which permits growth on acetate as a sole carbon and energy source. However, glycerol did not promote excretion of acetate aerobically. Under microaerobic conditions, cell growth was restricted concurrently, accompanied by excretion of lactate, acetate, formate, and ethanol (and possibly succinate, though not shown in the figure).

Tables 2.1–2.4 show the enzyme activities involved in the central metabolic pathways of *E. coli*, in which 26 enzymes are listed. Figure 2.2 shows the protein expression maps of 2DE for *E. coli* grown under different conditions. As can be seen from the figure, many proteins were induced or repressed either when the DO level was varied from aerobic to microaerobic conditions in a glucose medium, or the carbon source was changed from glucose to acetate, gluconate, or glycerol under aerobic conditions. In order to see how the protein expression levels changed under different culture conditions, the abundances of each protein under different culture conditions were divided by the corresponding one in the control experiment, such as aerobic conditions using glucose as the sole carbon source. Those ratios are shown in the metabolic pathway maps of Figure 2.3. The thickness of the arrow depends on the magnitude of the change in the protein expression level. Let us analyze the results one by one in the next section.

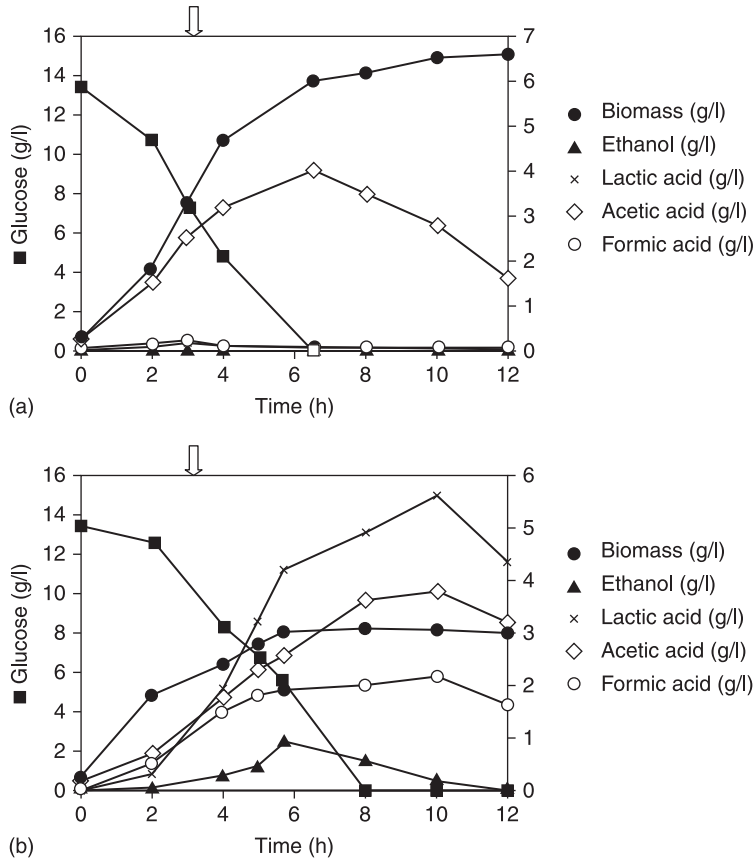

Figure 2.1 **Batch cultivations of *E. coli* K12 using different DO levels and different carbon sources: (a) glucose under aerobic conditions; (b) glucose under microaerobic conditions; (c) gluconate under aerobic conditions; and (d) glycerol under aerobic conditions. The arrows indicate that *E. coli* cells were harvested for the proteome analysis and enzyme activity measurement**

2.2.2 Metabolic regulation by protein expressions

Glycolysis and anaplerotic pathway

An early investigation showed that glucose transport genes exhibited high basal expression, and *ptsHI* genes were positively stimulated by the

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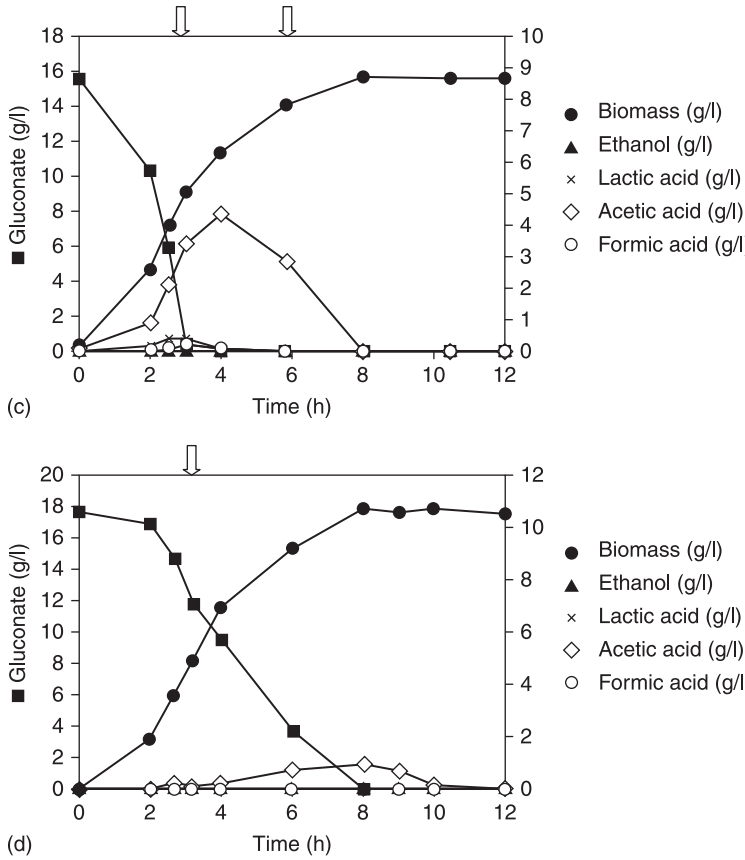


Figure 2.1 *Continued*

cAMP-CRP receptor protein and also by the growth on glucose, while *crr* promoters within *pts* may be negatively regulated by cAMP-CRP (Danchin, 1988; Fox et al., 1992). Plumbridge (1999) reported that these genes are in the same operon, and the operon is known to be regulated by Mlc. In the absence of glucose, Mlc represses the operons. In the presence of extracellular glucose, the conformation of $EIIBC^{Glc}$ protein changes and bonds strongly with Mlc, which no longer represses the operon. However, an essential feature of the phosphotransferase system (PTS) is that the phosphoryl donor molecule is PEP and not ATP (the regulation of PTS is explained in more detail in Chapter 3). The experimental results show that the glucose transport genes *ptsHI-crr* were slightly up-regulated under microaerobic conditions in comparison to the aerobic growth on glucose, and

Table 2.1 Activities of glycolytic and anaplerotic enzymes in response to carbon sources and DO levels. Ratio calculation was based on the control of aerobic growth on glucose

C source	Glucose				Acetate		Gluconate		Glycerol	
DO level	Aerobic		Microaerobic		Aerobic		Aerobic		Aerobic	
Enzyme	Activity	Control	Activity	Ratio	Activity	Ratio	Activity	Ratio	Activity	Ratio
PTS	0.015±0.001	1	0.017±0.00	1.131	ND	–	ND	–	ND	–
Hxk	0.032±0.00	1	0.039±0.00	1.221	0.022±0.002	0.71	0.027±0.002	0.85	0.014±0.002	0.44
Pgi	3.29±0.05	1	4.66±0.04	1.41	2.88±0.02	0.62	2.86±0.05	0.87	2.18±0.04	0.66
Pfk	0.34±0.01	1	0.54±0.02	1.64	0.19±0.02	0.56	0.17±0.02	0.70	0.22±0.01	0.64
Fbp	0.024±0.00	1	0.033±0.00	1.371	0.052±0.001	2.16	0.12±0.02	5.00	0.077±0.001	3.21
Fba	1.60±0.01	1	1.92±0.01	1.20	0.93±0.02	0.58	1.35±0.04	0.84	1.02±0.02	0.66
Tpi	2.80±0.02	1	3.50±0.02	1.25	2.27±0.02	0.81	3.57±0.02	1.02	4.14±0.02	1.48
GAPDH	0.036±0.001	1	0.051±0.00	1.402	0.018±0.001	0.51	0.039±0.002	1.05	0.045±0.001	1.25
Pgk	0.061±0.01	1	0.080±0.01	1.32	0.28±0.01	0.46	0.068±0.01	1.11	0.076±0.02	1.25
Pyk	0.054±0.00	1	0.084±0.00	1.561	0.016±0.001	0.29	0.058±0.001	1.07	0.036±0.002	0.67
Ppc	0.22±0.02	1	0.13±0.01	0.59	0.050±0.001	0.23	0.26±0.02	1.17	0.40±0.01	1.82
Pck	0.037±0.00	1	0.040±0.00	1.083	0.11±0.03	2.97	0.066±0.003	1.78	0.047±0.004	1.29
Mez	ND	–	ND	–	0.008±0.001	–	ND	–	ND	–

Table 2.2 Activities of PP and E-D pathway enzymes in response to carbon sources and DO levels. Ratio calculation was based on the control of aerobic growth on glucose

C source	Glucose				Acetate		Gluconate		Glycerol	
DO level	Aerobic		Microaerobic		Aerobic		Aerobic		Aerobic	
Enzyme	Activity	Control	Activity	Ratio	Activity	Ratio	Activity	Ratio	Activity	Ratio
G6PDH	0.35±0.02	1	0.22±0.01	0.64	0.14±0.01	0.40	0.50±0.01	1.42	0.44±0.01	1.25
6PGDH	0.28±0.02	1	0.21±0.01	0.74	0.19±0.02	0.68	0.45±0.02	1.61	0.33±0.02	1.18
E-D Pathway	0.45±0.02	1	0.15±0.02	0.33	0.26±0.01	0.57	3.01±0.02	6.69	0.38±0.04	0.85

Table 2.3 Activities of fermentative enzymes in response to carbon sources and DO levels. Ratio calculation was based on the control of aerobic growth on glucose

C source	Glucose				Acetate		Gluconate		Glycerol	
DO level	Aerobic		Microaerobic		Aerobic		Aerobic		Aerobic	
Enzyme	Activity	Control	Activity	Ratio	Activity	Ratio	Activity	Ratio	Activity	Ratio
Ack	0.48±0.02	1	0.75±0.01	1.56	0.34±0.02	0.70	0.55±0.04	1.15	0.13±0.02	0.27
LDH	0.66±0.05	1	1.22±0.04	1.85	0.34±0.03	0.51	1.01±0.05	1.53	0.61±0.04	0.92
ADH	0.005±0.002	1	0.062±0.04	12.40	ND	–	0.009±0.002	–	0.005±0.002	–
Pfl	ND	–	0.021±0.02	–	ND	–	ND	–	ND	–

Table 2.4 Activities of TCA cycle and glyoxylate shunt enzymes in response to carbon sources and DO levels. Ratio calculation was based on the control of aerobic growth on glucose

C source	Glucose				Acetate		Gluconate		Glycerol	
DO level	Aerobic		Microaerobic		Aerobic		Aerobic		Aerobic	
Enzyme	Activity	Control	Activity	Ratio	Activity	Ratio	Activity	Ratio	Activity	Ratio
CS	0.051±0.000	1	0.0076±0.0001	0.15	0.25±0.01	4.90	0.32±0.01	6.27	0.23±0.02	4.51
ICDH	1.15±0.02	1	0.14±0.02	0.12	0.26±0.02	0.23	2.20±0.04	1.91	1.88±0.04	1.63
Icl	0.013±0.002	1	0.006±0.002	0.46	0.12±0.02	9.23	0.019±0.001	1.46	0.017±0.003	1.31
KGDH	0.022±0.002	1	ND	–	0.058±0.001	2.91	0.060±0.001	2.73	0.065±0.001	2.95
Fum	0.061±0.004	1	0.017±0.002	0.28	0.20±0.01	3.27	0.11±0.01	1.80	0.10±0.01	1.64
MDH	0.056±0.00	1	0.030±0.001	0.54	0.15±0.01	2.68	1.78±0.01	1.96	0.10±0.01	1.78

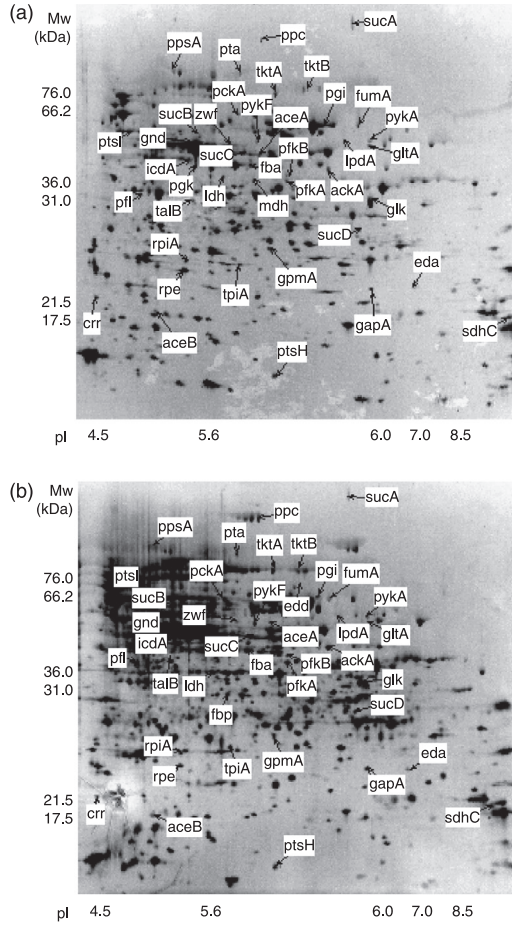
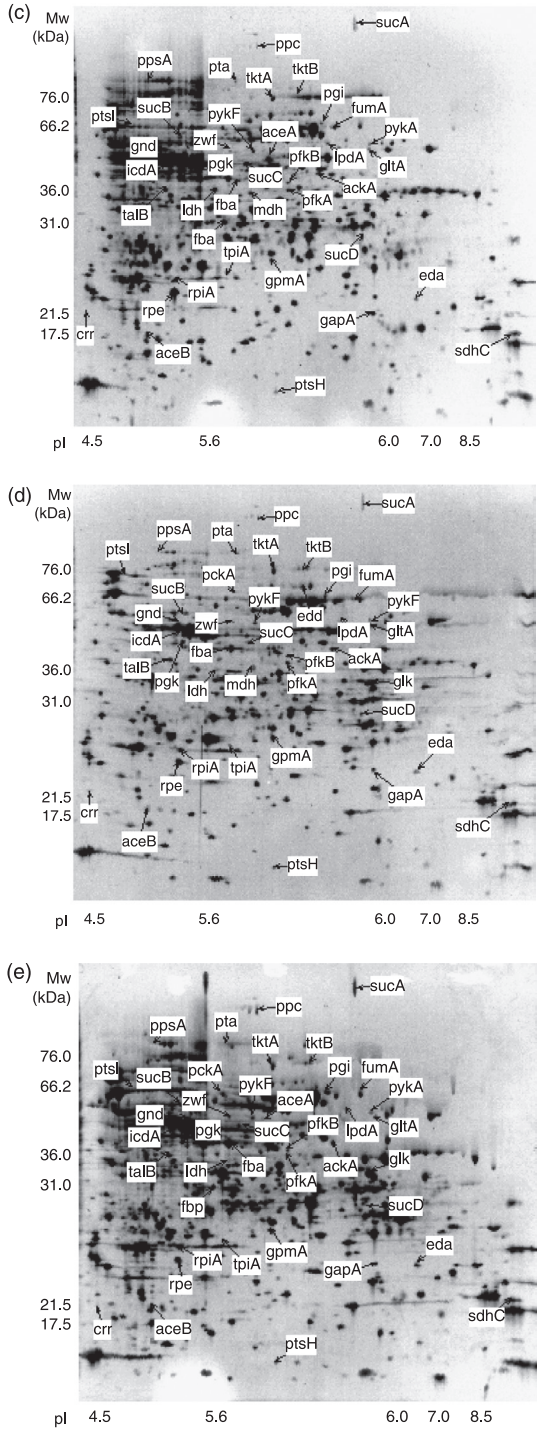


Figure 2.2 2-DE gel maps of the total lysate of *E. coli* cells grown on: (a) glucose under aerobic conditions; (b) glucose under microaerobic conditions; (c) acetate under aerobic conditions; (d) gluconate under aerobic conditions; and (e) glycerol under aerobic conditions.



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significantly down-regulated by 2- to 5-fold when the carbon source was changed to acetate, gluconate, or glycerol. The enzyme activity of PTS was only detected in glucose medium (Table 2.1), confirming the efficient induction by glucose either aerobically or microaerobically. Glk encoding the first enzyme glucokinase, which drives glucose entry into the glycolytic pathway in the cell, was kept relatively unregulated by not more than about 1.2-fold in all cases.

Most of the protein levels for the glycolysis genes, such as *pgi*, *pfkA*, *fba*, *gapA*, *pgk*, *eno,* and *pykF* were also observed to be up-regulated under microaerobic conditions, indicating a 'stepping-up' of anaerobic glucose utilization via glycolysis. The rapid fall of TCA cycle enzyme activities (Table 2.4) and the significant increase in fermentative enzyme activities (Table 2.3) under microaerobic conditions, indicate that glucose utilization was switched toward fermentation under microaerobic conditions.

In contrast, the protein levels for the common glycolysis genes *pgi*, *pfkA*, *fba*, *gapA*, *eno*, *pykF*, and anaplerotic pathway gene *ppc*, were highly repressed by about 2- to 4-fold in acetate medium compared to those in glucose medium. Simultaneously, the protein levels of the gluconeogenic pathway genes *fbp*, *pckA*, and *ppsA* were highly induced by about 2.5-fold, 3.7-fold, and 8.3-fold, respectively, which were thought to be subjected to positive regulation by the catabolite repressor/activator Cra, a global catabolite repression regulator (Saier and Ramseier, 1996), formerly known as the fructose repressor FruR, a global regulator concerned with carbon utilization by transcriptional modulation of the target genes (Ramseier et al., 1995). The enzyme activities given in Table 2.1 agree with the fact that carbon flow will be channeled through the gluconeogenic pathway when acetate is metabolized, and the flux in gluconeogenic direction is much smaller than the glycolytic flux during growth on glucose. Note that in the reaction between F6P and F1, 6BP, PEP and PYR, PEP and OAA, the involved enzymes are tightly regulated. The protein level of *pfkA*, encoding for the main phosphofructokinase, Pfk-1, was repressed by about 2-fold, while *pfkB*, encoding for the minor phosphofructokinase Pfk-2, was kept nearly unchanged. Correspondingly, the activity of Pfk dropped 1.8-fold in acetate medium, while *fbp* gene was up-regulated by 2.5-fold, and the enzyme activity of FBPase varied in a co-ordinate manner, and increased by 2.2-fold (Table 2.1). It has been proved that *pfkA* is subject to the regulation by the catabolite repressor/activator Cra (FruR), while the enzyme Pfk-1 is allosterically activated by ADP and inhibited by PEP and ATP. The inhibitory effect of ATP is opposed

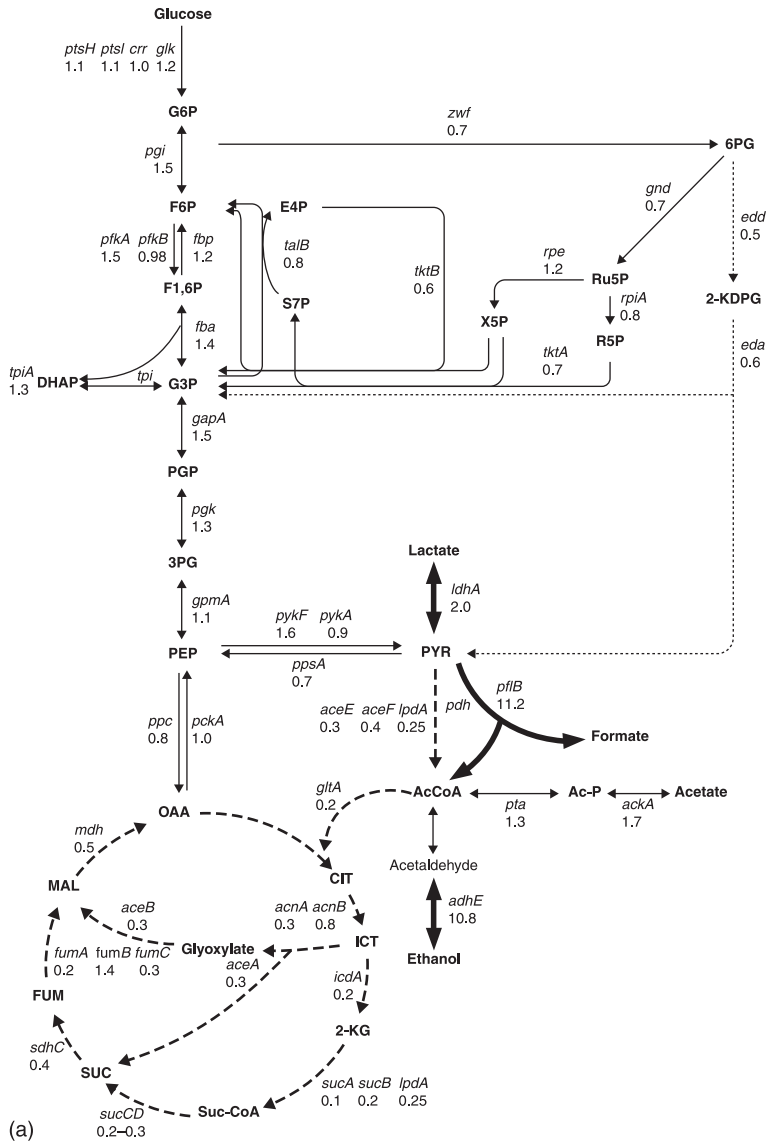

Figure 2.3 The relative expression levels of *E. coli* K-12 proteins of central metabolic pathways under different conditions based on 2DE results: (a) microaerobic growth on glucose; (b) aerobic growth on acetate; (c) aerobic growth on gluconate; and (d) aerobic growth on glycerol. Aerobic growth on glucose was used as the control.

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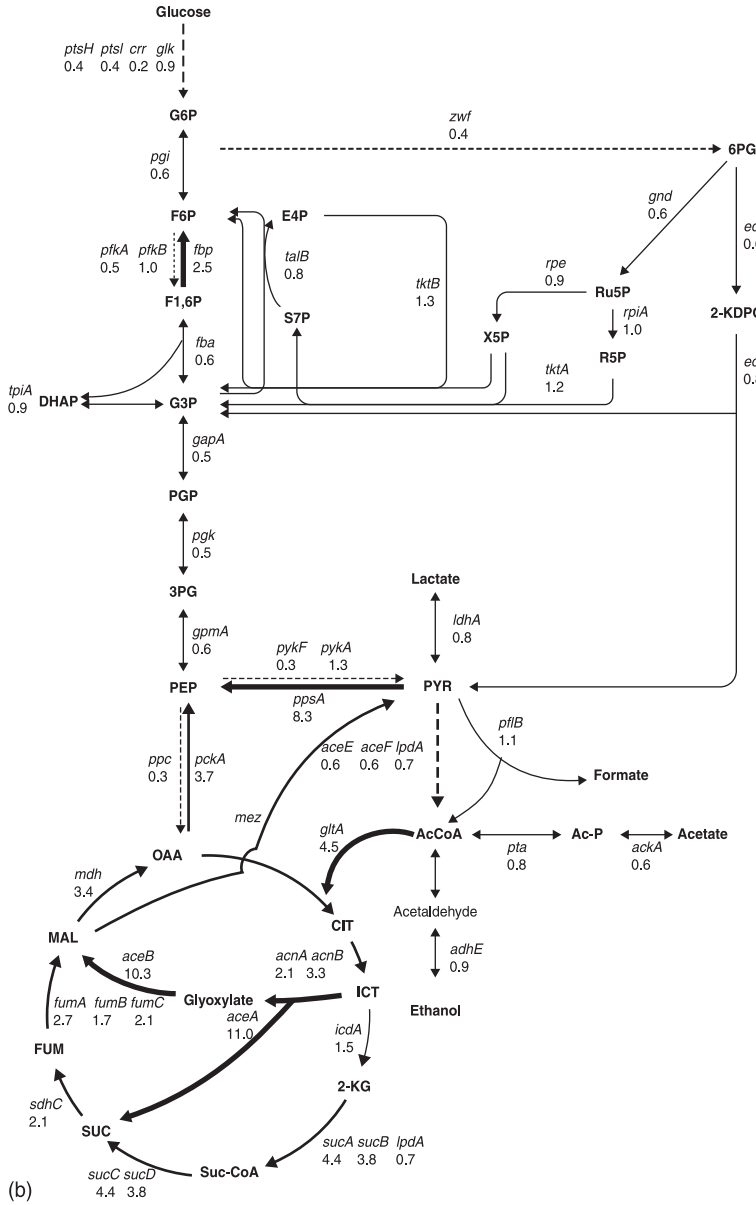

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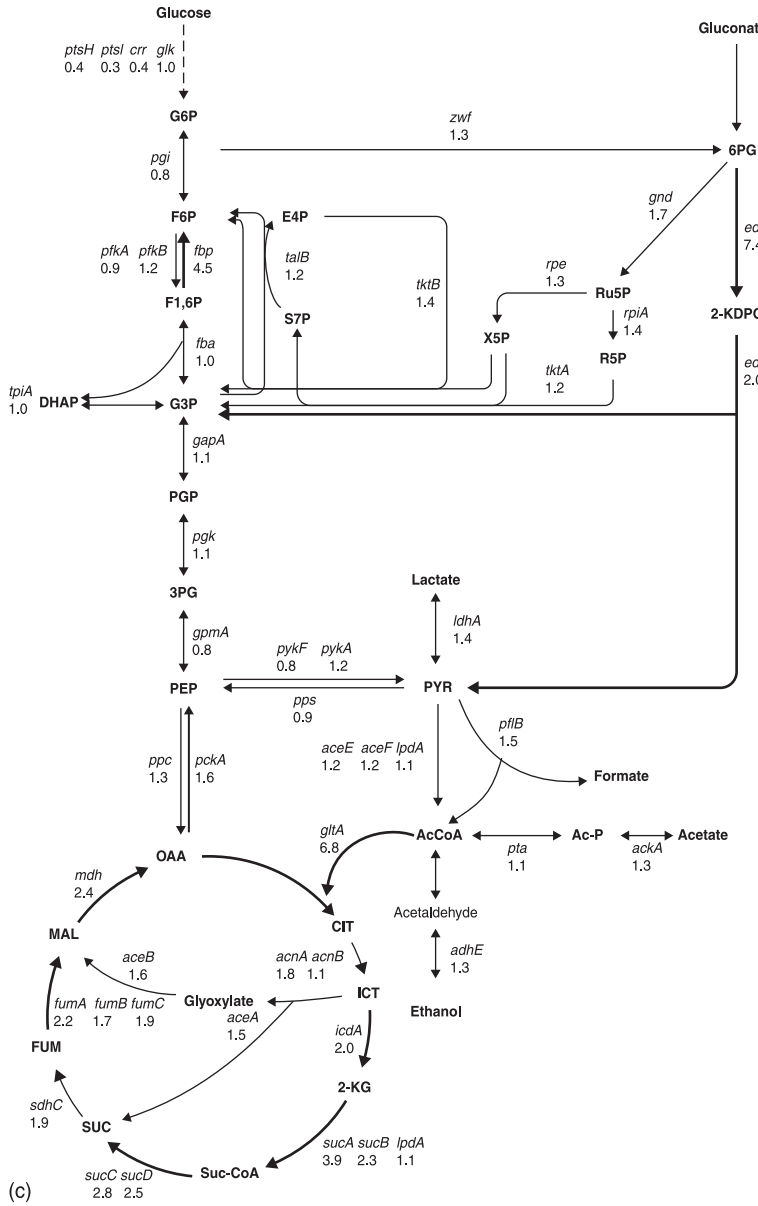

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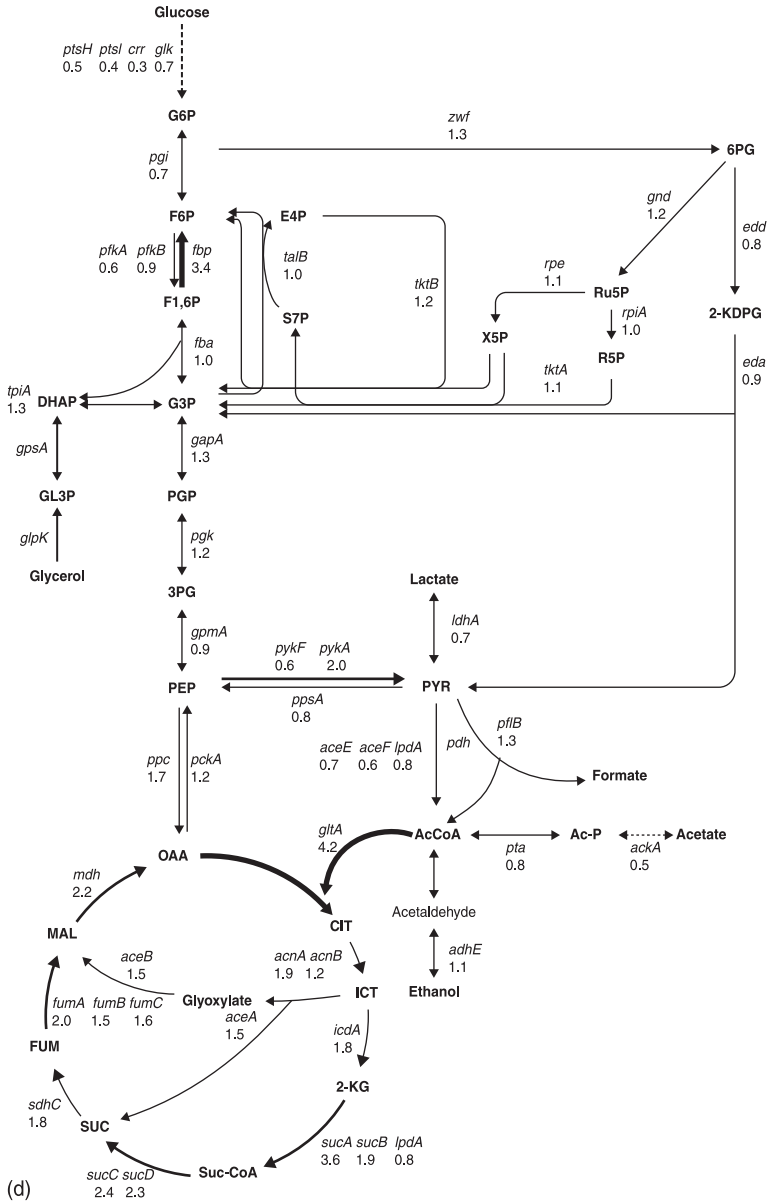

Figure 2.3 *Continued*

by AMP, and intensified by citrate, which acts as a signal of the availability of alternative sources of ATP (Martin, 1987). This control might link to an increase in the rate of Pfk-1 synthesis under anaerobic conditions. Pfk-2, insensitive to ATP inhibition, may serve as another role (i.e. maintain the futile cycle for the regulatory amplification) (Campos et al., 1984; Guixe and Babul, 1985). The down-regulation of PykF, but not PykA, suggests that these two isoenzymes were differentially regulated. Indeed, *pykF* is negatively regulated by Cra (FruR).

The protein level of *ppc* gene was down-regulated by 3-fold in the acetate medium. This result is consistent with the direction of the metabolic flux. The carbon flux from oxaloacetate (OAA) to phosphenolpyruvate (PEP) will be dominant in such a case, and is catalyzed by Pck. In fact, the protein level of *pckA* gene was up-regulated by 3.7-fold, which is also modulated by Cra at the transcriptional level. The enzyme activities were coordinately regulated, and Pck increased by nearly 3-fold, while Ppc decreased by 4.3-fold (Table 2.1). High protein level expressions of both *ppc* and *pckA* led to futile cycling. Moreover, the protein level of *ppsA* gene was also found to be highly induced by about 8.3-fold in acetate medium. In addition, malic enzyme, Mez, which converts malate to pyruvate, showed some activity only in acetate medium, and was not detectable for other cases. This result suggests that Pps and Mez may play an important role for the gluconeogenic flux during the metabolism of acetate. In fact, the induction of *ppsA* and *maeB* genes of *E. coli* during growth in acetate medium has been confirmed by DNA microarray (Oh and Liao, 2000; Oh et al., 2002).

In contrast, most of the glycolytic genes were not affected significantly during growth in gluconate medium. An exception is the *fbp* gene, which was induced by 4.5-fold in protein level. The enzyme activity of FBPase increased 5-fold in gluconate medium (Table 2.1). The high induction of the *fbp* gene will drive part of GAP from the ED pathway through gluconeogenesis to supplement G6P. Surprisingly, the protein level of *pckA* gene was found to be induced by 1.6-fold, and the enzyme activity of Pck varied accordingly (Table 2.1). The same phenomenon with *pckA* appeared in cells grown on glycerol. However, this induction is unexpected, since the flux in Pck-mediated reaction is not needed in both cases. The *pckA* may be induced gratuitously by the increased level of cAMP during growth in non-glucose medium. It has been reported that the concentration of cAMP in glycerol-grown cells was much higher than that in glucose-grown cells of *E. coli* (Epstein et al., 1975; Unden and Duchene, 1987). During growth on glycerol, one significantly affected

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gene was *pykA*, which was induced by 2-fold at protein level. When *E. coli* was cultivated in glycerol, the flux from PEP to pyruvate is governed by Pyk rather than the PTS. Therefore, it is reasonable to expect that one of the *pyk* genes is up-regulated to satisfy the significantly increased demand for the flux from glycolysis. Apparently, the *pykA* gene rather than *pykF* serves this role. Since Pyk is activated at the protein level by F1, 6BP, whose concentration is relatively low, is not a good indicator of the metabolic state during growth in glycerol (Lowry et al., 1971). Therefore, *pykF* may remain almost inactive due to the low concentration of its allosteric activator. *PykA*, however, is activated by cAMP, which is higher in glycerol medium than in glucose medium (Epstein et al., 1975; Unden and Duchene, 1987), and its activity can still be mediated at the protein level. Therefore, *pykA* is the better choice during growth on glycerol.

Pentose phosphate pathway and Entner-Doudoroff pathway

The two enzymes involved in the oxidative PP pathway, glucose 6-phosphate dehydrogenase (G6PDH) encoded by *zwf* gene and 6-phosphogluconate dehydrogenase (6PGDH) encoded by *gnd* gene, are down-regulated during microaerobic growth in glucose medium and aerobic growth in acetate medium. However, both enzymes are up-regulated when the cells grow in gluconate or glycerol mediums. The enzyme activities varied in a coordinated manner (Table 2.2).

The ED pathway is significantly induced when grown on gluconate, based on the measurements of the overall activity of the ED enzymes and the 2DE result. Table 2.2 shows the activities of the two ED pathway enzymes in *E. coli*. Both enzymes are present at high levels during growth on gluconate, where the protein level of the *edd* gene was highly up-regulated by 7.4-fold, and *eda* was induced by 2-fold, as compared to the case of using glucose as the carbon source, and the overall activity of ED pathway enzymes coordinately increased by about 7-fold (Table 2.2). Note that the overall activity of the ED enzymes, including 6PG dehydratase (edd) and KDPG aldolase (eda), was assayed by measuring 6PG-dependent formation of pyruvate, which is determined colorimetrically as its dinitrophenylhydrazone (Esienberg and Dobrogosz, 1967; Lessie and Whiteley 1969). This assay procedure underestimates the level of KDPG aldolase, which is usually present in excess compared with 6PG dehydratase. It measures the rate-limiting component in the pathway, 6PG dehydatase.

Both enzymes G6PDH and 6PGDH were known to be subject to cellular growth rate regulation, which is proportional to the growth rate influenced by the medium (Wolfe et al., 1979; Pease and Wolfe, 1994). Indeed, the cell growth was slow under microaerobic conditions and in acetate medium, while it was faster during growth on gluconate and glycerol as well as the cell growth on glucose. In addition, an early study reported that 6PGDH was induced by gluconate (Lowry et al., 1971). The other genes involved in non-oxidative metabolism, such as *rpe*, *rpi*, *tal*, and *tkt*, did not differ significantly between growth conditions. It is known that the *edd* gene, containing a regulatory region, is induced by gluconate, and the *eda* gene is probably induced by EDGP, the product of *edd* (Sugimoto and Shiio, 1987; Conway, 1992), and both genes are subjected to negative control by Cra. This result indicates that the ED pathway is predominant in dissimilation of gluconate in *E. coli*, while insignificant in the metabolism of acetate, glucose, or glycerol.

Fermentative pathway

Among the fermentative genes, the protein levels of *pfl* and *adhE* genes were dramatically induced by about 11.2-fold and 10.8-fold, respectively, in response to the shift from aerobic to microaerobic conditions in glucose medium (Figure 2.2). The enzyme activity of Pfl was only detected in microaerobic conditions, and the activity of NADH-dependent ADH also increased by 12.4-fold under microaerobic conditions (Table 2.3).

Regulation of *pfl* synthesis and activity was subjected to control by the Fnr and ArcA/B two-component regulatory systems. Fnr, an oxygen-sensing global regulator, which is an iron sulfur-dependent DNA-binding protein and recognizes a specific sequence motif found in the promoter regions of the genes it regulates, serves as an activator of the transcription of anaerobically regulated genes (Gunsalus and Park, 1996; Kiley and Beinert, 1999). The significant anaerobic induction of the *pfl* operon was thought to account for Fnr and ArcA/B, which mediate the residual transcriptional activation of the operon (Alexeeva et al., 2000; Sawers and Suppmann, 1992). However, the expression of *pfl* was little changed in acetate, gluconate, or glycerol medium, although this fermentative pathway is not used when growing aerobically in acetate medium (Sawers and Suppmann, 1992). Expression of *adhE i*s also strongly induced by micro-aerobiosis. However, this induction is independent of the Fnr and ArcA transcription factors. There

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appears to be a direct correlation between the NADH/NAD^+ ratio and enzyme synthesis; the higher the ratio, the more ADH is synthesized. Moreover, the ADH protein itself may exert a regulatory function, since the gene expression was enhanced dramatically in the *adhE* mutant (Bock and Sawers, 1999). Therefore, the induction of *adhE* is not surprising, since the previous study showed that the significant differences of the NADH/NAD^+ ratios between aerobic and anaerobic cultures of 0.02 and 0.75, respectively, have been observed as the DOT (dissolved oxygen tension) of the culture was decreased (Alexeeva et al., 2000). The *ldhA* gene, encoding NAD^+ linked enzyme LDH, was also highly enhanced by 2-fold during microaerobic fermentation of glucose. This result is consistent with the study by Mat-Jan et al. (1989), who found that the fermentative LDH was cojointly induced by anaerobic conditions and acidic pH (Alexeeva et al., 2000). Since *E. coli* satisfies energy requirements through glycolysis at the accelerated rate under anaerobic conditions, the NADH produced must be re-oxidized to NAD^+, which is required to maintain glycolysis since it is again the substrate for GAPDH in glycolysis. In the presence of oxygen, the oxidation of NADH occurs through molecular oxygen in the respiratory chain, while in the absence of oxygen it proceeds through reduction of an organic acid, and thereby the conversion of pyruvate to lactate by LDH is preferable. The LDH activity changed in response to carbon sources. Namely, it was repressed in acetate, slightly enhanced in gluconate, and almost unchanged in glycerol.

The protein levels of *pta* and *ackA* genes, involved in the reversible acetyl CoA metabolism in the cell, are up-regulated by 1.3-and 1.7-fold, respectively, in the case of microaerobic conditions (Figure 2.4). The enzyme activity of Ack in microaerobically grown cells was 1.56 times as high as that grown aerobically in glucose medium (Table 2.3). The *pta* and *ackA* genes are constitutively expressed and present in the same operon, but were regulated differentially through different promoters. It has been proposed that the intermediate of this pathway, acetyl phosphate, might be an important effector of gene regulation, while the levels of acetyl phosphate vary dramatically, depending on the carbon source in the growth medium. For example, in the defined medium under limiting phosphate concentrations, very low levels of acetyl phosphate were observed when cells were grown on glycerol (<40 mmol/l), moderate levels on glucose (300 mmol/l), and high levels on pyruvate (Wanner and Wilmers-Riesenberg, 1992; McCleary and Stock, 1994). During growth on glycerol, Ack is down-regulated by almost 3-fold (Table 2.3).

This result is consistent with the observation that acetate production in glycerol medium is much lower than in glucose medium. However, a relatively small change of *pta* was observed. It may perhaps ensure the production of acetyl phosphate for regulatory purposes. During growth on acetate, both *pta* and *ackA* were down-regulated. This result suggests that other genes may be responsible for acetate uptake during growth on acetate. Indeed, it was proposed that the *acs* gene (coding for acetyl-CoA synthetase) is mainly responsible for acetate uptake, whereas the *pta-ackA* pathway is used for acetate excretion during growth on glucose (Oh et al., 2002). In gluconate medium, both genes were observed to be up-regulated. The measured enzyme activities of Ack were roughly correlated with the protein expression levels.

TCA cycle and glyoxylate shunt

It was observed that there was a significant effect of the change in either oxygen level or carbon source on the TCA cycle genes. Under microaerobic conditions, the TCA cycle protein expressions fell by around 2- to 10-fold, as compared to those under aerobic conditions. In contrast, if acetate, gluconate, or glycerol was used as the carbon source instead of glucose, most of the TCA cycle genes were up-regulated by 2- to 6-fold.

Citrate synthase (CS), encoded by the *gltA* gene, the first enzyme to enter the TCA cycle, was suppressed by oxygen limitation and excess glucose, but elevated by oxygen (5-fold) and other oxidized carbon sources (4- to 7-fold), such as acetate, gluconate, or glycerol. The α-ketoglutarate dehydrogenase (KGDH) complex was encoded by *sucA* and *sucB*, and shared the third gene *lpdA* with PDH complex. These genes were strongly suppressed by oxygen limitation and induced by acetate and other oxidized carbon sources. Indeed, the enzyme activity of KGDH was not detectable in glucose medium under microaerobic conditions (Table 2.4). These observations coincide with an early study of Amarasingham et al. (1965). At the important control point of the junction between the TCA cycle and the glyoxylate shunt, *aceA* and *aceB* were cojointly induced by more than 10-fold in acetate medium. The enzyme activity of isocitrate lyase (Icl) increased by more than 9-fold in acetate medium (Table 2.4). The highly regulated glyoxylate shunt was absolutely essential to direct the carbon flow through this bypass to generate a 4-carbon precursor for biosynthesis in acetate metabolism (Chung et al. 1988).

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The TCA cycle enzymes (Table 2.4) are shown to be mainly dependent on the gene expression levels. These genes are known to be subjected to cAMP-Crp mediated catabolite repression and ArcA, Fnr, and SoxRS oxidative stress regulatory system mediated aerobic-anaerobic repression (Cronan and Laporte, 1999). Although there was a general reduction in TCA cycle gene expression and enzyme activity related to microaerobic conditions in glucose medium, the variations of individual genes were not coordinated, implying that these genes are subjected to different regulations and different regulatory mechanism. For instance, *fumA*, *fumB*, and *fumC* are regulated differently by oxygen availability, where *fumB* is slightly abundant under microaerobic conditions (1.4-fold) (Figure 2.4), since the expression of the *fumB* gene is controlled by anaerobic transcriptional activator, Fnr, whereas the expressions of *fumA* and *fumC* decreased 3- to 5-fold when the oxygen level was reduced to microaerobic conditions as compared to aerobic conditions (Figure. 2.4). It was proposed that anaerobic expression of *fumA* promoter was repressed by ArcA and Fnr, while expression of *fumC* was repressed by ArcA only, but required the SoxRS regulatory protein, which is a positive regulator for controlling synthesis of proteins in response to oxidative stress (Park and Gunsalus, 1995; Tseng et al., 2001). In addition, the observation of 4-fold higher expression of *fumA* than that of *fumC* suggested that *fumA* is predominant under aerobic conditions. However, *fumC* is relatively less affected by the type of carbon source than *fumA*, indicating that *fumA* is shown to be under catabolite control.

The *acnA* shows 2- to 3-fold lower expressions due to catabolite and anaerobic repression, but *acnB* expression is relatively constant, although 3-fold up-regulated in acetate medium, since *acnB* is used preferably for the glyoxylate shunt, indicating that *acnB* was regulated differently. The *acnA* appeared to be activated by the SoxRS and Fnr (Cronan and Laporte, 1999). It was demonstrated that ArcA functions as a repressor of *gltA* expression during both aerobic and anaerobic growth through a *lacZ* fusion study (Park et al., 1994). The relative pattern of carbon control by *mdh*, *gltA*, and *sdhC* gene expressions are similar. However, the magnitude of oxygen control of *mdh* and *gltA* gene expressions were larger than that of *sdhC*. The difference in magnitude may reflect the function of the *mdh* and *gltA* protein under both aerobic and anaerobic conditions, whereas the *sdhCDAB* proteins are primarily used for aerobic cell growth (Park et al., 1995; Gunsalus and Park, 1996). The protein level of *icdA* gene encoding isocitrate dehydrogenate (ICDH), competing for the common substrate

with isocitrate lyase at the node, is up-regulated by 1.5-, 2.0-, and 1.8-fold in acetate, gluconate, and glycerol mediums, respectively. Nevertheless, the activity of ICDH did not agree with this result in the case of acetate, which reduced by nearly 4.3-fold (Table 2.4). This fact may be explained as follows: the *icdA* gene was induced by acetate, and in turn led to an increased amount of the enzyme protein. However, in order to prevent the excess flux of isocitrate through ICDH, the enzyme activity was regulated by reversible inactivation of ICDH, which is catalyzed by bifunctional kinase/phosphatase, encoded by *aceK*, since the phosphorylated form of ICDH has no activity (Holms, 1988). Although regulation of the enzyme ICDH and Icl activities are opposite in acetate medium, the absolute enzyme activity of ICDH, which leads the carbon flow to the TCA cycle, is still dominant over the glyoxylate bypass directing enzyme, Icl (Table 2.4), because of the balance of the rate of supply of NAD(P)H and ATP with the demand for biosynthesis.

Comparison of the protein expression level and enzyme activity

Figure 2.4 shows the correlation of the protein expression levels and the corresponding enzyme activities. The logarithmic ratio of the enzyme activity is plotted versus the logarithmic ratio of the protein expression level. As can be seen in Figure 2.5, most of the spots are close to the 45° line, except ICDH under aerobic acetate conditions, indicating that protein abundances revealed by 2DE are correlated, to a large extent, with enzyme activity. However, it is obvious that there exist deviations from the linear correlation between the logarithmic protein expression ratios and the enzyme activity ratios. The deviations may be caused by the fact that protein products expressed from a single gene can migrate to multiple spots on 2DE gels, and also the proteins from multiple genes can run to the same coordinates on a gel, because of differential protein processing and posttranslational or artifactual modifications (Gygi et al., 2000a,b). Both differential migration and co-migration of proteins complicate comparative and quantitative pattern analysis of 2DE gels.

However, although the enzyme activity is primarily determined by the protein expression level, subtle regulations are exerted by many effectors at the enzyme level to satisfy the need for metabolism. ICDH in acetate medium may be such a case, which was regulated by reversible inactivation catalyzed by bifunctional kinase/phosphatase, as discussed

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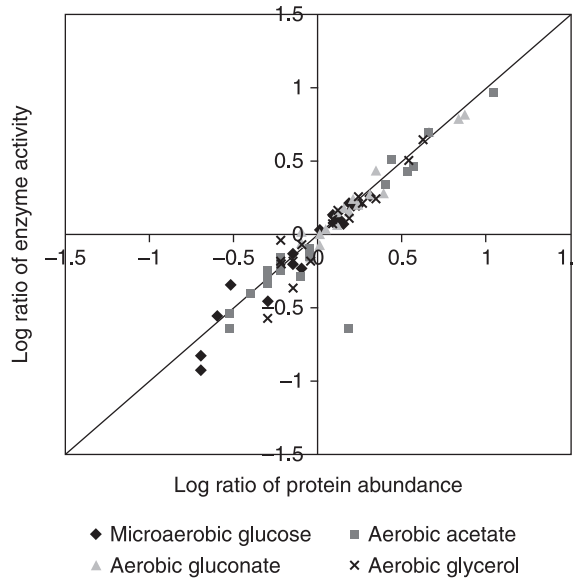
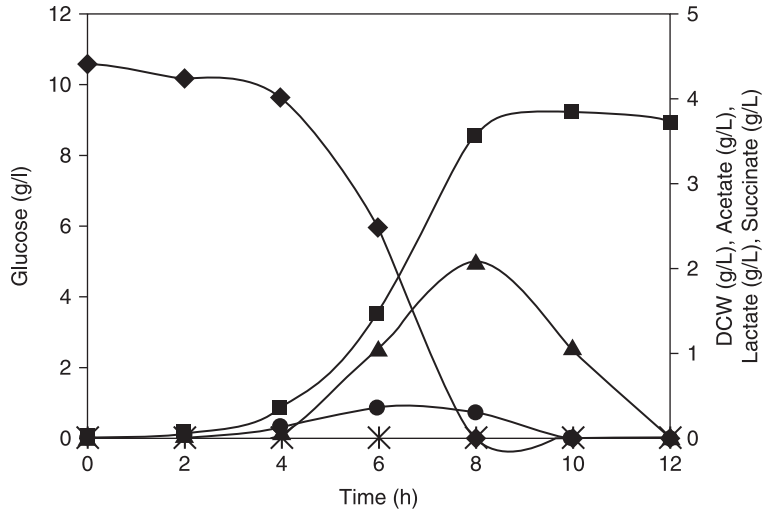


Figure 2.4 **Comparison of the logarithmic protein expression ratios based on 2DE and the corresponding enzyme activity ratios. Data were taken from Tables 2.1–2.4 and Figure 2.3. Each set of data was plotted on the figure by the logarithmic ratio value**

above. Moreover, the Ppc and Pck enzymes, involved in the anaplerotic and gluconeogenic pathways, are also subject to fine control by some effectors.

2.3 Metabolic regulation during the time course of batch culture

Let us now consider how metabolism changes with respect to time in the batch culture of *E. coli* (Rahman et al., 2007, 2008). The growth curve of *E. coli* BW25113, as shown in Figure 2.5, indicates that the exponential growth of the bacteria continued until about 7.5 h, followed by a short period of growth before the cells entered into the stationary phase. The end of the exponentially growing phase was correlated with almost complete utilization of glucose by the bacteria from the extracellular medium. Therefore, samples collected at 6, 8, and 12 h for gene expression



Maximum specific growth rate (m)	0.54 ± 0.03
Specific glucose uptake rate (mM/gDCW/h)	5.52 ± 0.06
Yield of acetate on glucose (g/g)	0.2 ± 0.05
Biomass yield (g DCW/g substrate)	0.36 ± 0.03

Figure 2.5 Growth curves of *E. coli* BW25113, which was grown in minimal media containing 10 g glucose/l as the sole carbon source. Fermentations were carried out at pH 7, at 37°C, with an agitation speed of 350 rpm to ensure 35–40% (v/v) of air saturation to maintain aerobic conditions of growth. Concentrations of ♦ glucose (g/l), ■ DCW (g/l), ▲ acetate (g/l), ✳ succinate (g/l), ● lactate (g/l). Samples for gene expression analyses, enzyme activity measurement, and intracellular metabolite concentration measurements were collected at 6, 8, and 12 h of growth to represent exponential, early stationary, and stationary phases of growth, respectively

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and enzyme activity analyses represent the exponential, early stationary, and stationary growth phases, respectively. Gene expression data obtained by RT-PCR were categorized under six global regulatory systems (*rpo*D, *rpo*S, *sox*RS, *fad*R/*icl*R, *cra*, and *arc*A). Figures 2.6a–f shows how the expression of the global regulatory genes changed, and how the metabolic pathway genes in these regulatory networks changed with respect to the growth phases.

The vegetative sigma factor RpoD regulates the expression of a wide range of genes during the exponential phase of growth (Jishage et al., 1996). Gene expression data indicate a slight decrease in the expression of *rpoD* and some of the *rpoD*-dependent genes, such as *pgi* and *ser*A, at the later phases of growth (Figure 2.6a). However, the expression of the general stress regulator *rpo*S increased as the culture proceeded from the exponential growth phase toward the stationary phase, complying with the reports on *rpoS* expression (Aronis, 2002b; Patten et al., 2004). In addition, the RpoS-regulated genes, such as *tktB*, *talA*, *acnA*, *sucA*, *fumC*, *acs*, and *sodC*, follow the expression pattern of *rpoS* with approximately 1.5 to 2-fold up-regulation at the later phases of growth, as compared to the expression levels at the exponential phase (Figure 2.6b). Of these genes, *fumC* and *acnA* are also regulated by the oxidative stress regulator, SoxRS. However, the expression of these TCA cycle genes is poorly correlated with the declining expression of *soxRS* from the exponential to the stationary phases of growth (Figure. 2.6c). This indicates that the expressions of *fumC* and *acnA* are predominantly affected by RpoS and not by SoxRS during the later phases of growth.

The induction of the stress regulator, *rpoS*, at the later growth phases is a well-known phenomenon (Aronis 2002; Weber et al., 2005). Expression of *soxRS* is induced by oxidative stress or by the reduction of NADPH/$NADP^+$ ratio (Liochev and Fridovich, 1992; Krapp et al., 2002). At the early stationary phase, although the synthesis of NADPH is expected to be decreased by the lower activities of the pentose phosphate (PP) pathway enzymes, such as G6PDH and 6PGDH, and the TCA cycle enzyme, ICDH, the NADPH level is likely to be maintained by the increased activity of the $NADP^+$-dependent malic enzyme encoded by *maeB* (a gluconeogenic enzyme), resulting in little effect on the NADPH/$NADP^+$ ratio (Table 2.5). Thus, the downward trend of *soxRS* expression at the later phases of growth could be related to the oxidative environment of the cells. The data suggest that SoxRS plays a major role during the exponential phase, when cells experience more oxidative stress resulting from the higher rate of respiration and higher specific growth rate. With the exception of *fumC* and *acnA*, which are related to

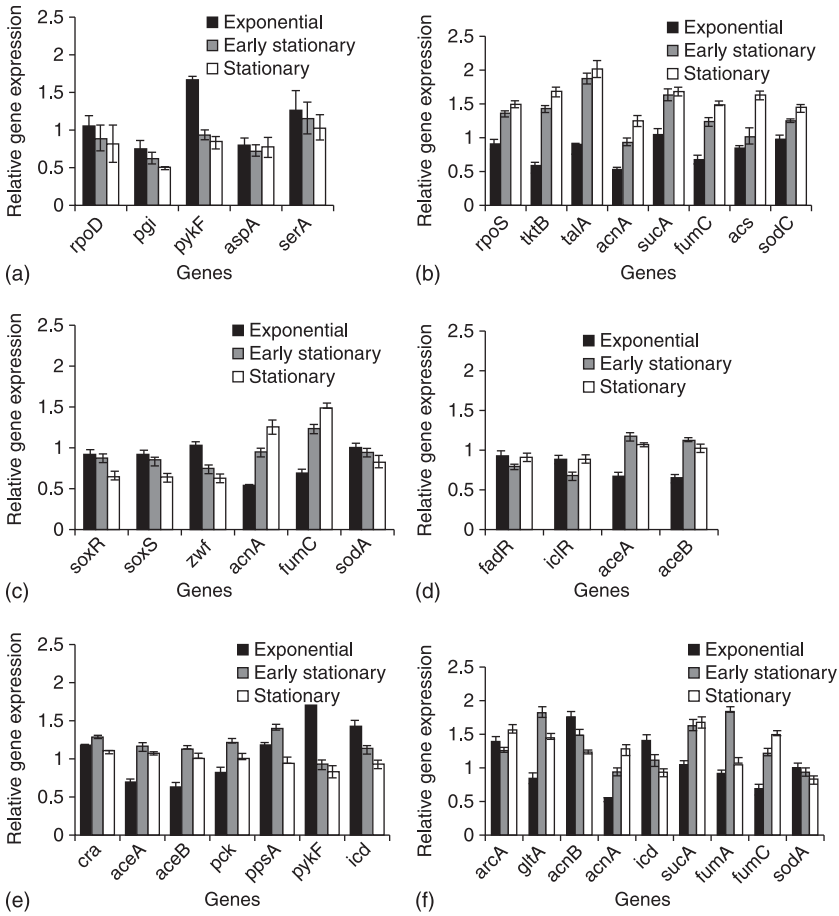

Figure 2.6 Relative gene expression of different global regulators and the metabolic pathway genes known to be regulated by those global regulators during different phases of growth. (a) Relative gene expression of *rpo*D and rpoD-regulated genes; (b) Relative gene expression of *rpo*S and rpoS-regulated genes; (c) Relative gene expression of *sox*RS and soxRS-regulated genes; (d) Relative gene expression of *fad*R/*icl*R and iclR-regulated genes; (e) Relative gene expression of *cra* and cra-regulated genes; and (f) Relative gene expression of *arc*A and arcA-regulated genes. Gene expression data were obtained by RT-PCR of samples collected at different phases of growth

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rpoS, the *soxRS*-dependent expression of *zwf* and *sodA* also supports this idea.

Figure 2.6b shows that *acs* is expressed in an *rpoS*-dependent manner during different phases of growth. The higher expression of *acs* accelerates acetate assimilation and increases biomass in the presence of acetate (Kumari et al., 2000; Lin et al., 2006). Again, acetate metabolism leads to the activation of the glyoxylate pathway that requires more AcCoA, as compared to the situation when AcCoA is only used up by the TCA cycle (Stryer, 1988). These facts are consistent with the observations of up-regulations of the glyoxylate pathway genes *aceA* and *aceB* and the low concentration of intracellular Ac-CoA, together with the higher expression of *acs* at the early stationary phase (Figures 2.6b and 2.7). However, *acs* expression shows further increase at the stationary phase, during which the glyoxylate pathway is down-regulated and the extracellular acetate concentration is almost negligible (Figure 2.5). Therefore, the results suggest that *acs* encoded AcCoA synthetase acts as the scavenger of extracellular acetate to maintain the intracellular concentration of AcCoA.

In addition to the RpoS regulated genes, an up-regulation is observed in several Cra regulated genes at the early stationary phase of growth, although the expression of *cra* itself was only slightly changed. A significant reduction of *pykF* expression is observed during the later phases of growth, which can be related to its repression by Cra, or because of its relation with the growth phase-dependent down regulation of *rpoD* (Bledig et al., 1996). The down-regulation of *icd* can be related to acetate metabolism and activation of the glyoxylate pathway (Cronan and LaPorte, 1996). The activities of the glyoxylate pathway enzymes Icl and MS are more than 5-fold higher at the early stationary phase of growth, and the gluconeogenic pathway genes *pck*, *pps*A, and *fbp* are also significantly increased (Figure 2.6e and Table 2.5). Higher activities of these enzymes led to the reduced level of intracellular AcCoA and PYR, while causing higher levels of OAA and PEP at the early stationary phase as compared to the exponential growth phase (Figure 2.7). The up-regulation of the glyoxylate pathway genes is related not only to extracellular acetate but also to its negative regulator IclR (Gui et al., 1996). The *fadR* and *iclR* changed in a coordinated manner, and their regulated genes, such as *aceA* and *aceB*, changed in the opposite direction, consistent with previous data (Figure. 2.6d) (Gui et al., 1996). The expression patterns of *aceA* and *aceB* are more related to IclR than to Cra (Figures 2.6d, e).

Figure 2.8 clearly shows how the iso-genes *tktA* and *tktB*, *talB* and *talA*, *acnB* and *acnA*, as well as *fumA* and *fumC*, backed up each other

Table 2.5 Specific activity of enzymes of *E. coli* metabolic pathways in minimal media under aerobic growth conditions at different phases of growth

Enzymes	EP (μmol/ min · mg protein)	ES (μmol/ min · mg protein)	STA (μmol/ min · mg protein)
Glycolytic pathway			
Pgi	2.36 ± 0.06	1.59 ± 0.05	0.77 ± 0.05
Pfk	0.93 ± 0.07	0.09 ± 0.04	0.043 ± 0.05
GAPDH	0.11 ± 0.06	0.016 ± 0.05	0.013 ± 0.05
Pyk	0.51 ± 0.04	0.16 ± 0.05	0.09 ± 0.04
Ppc	0.12 ± 0.05	0.054 ± 0.009	0.052 ± 0.06
Pentose phosphate pathway			
G-6PDH	0.33 ± 0.05	0.104 ± 0.04	0.098 ± 0.04
6-PGDH	0.28 ± 0.06	0.12 ± 0.05	0.102 ± 0.06
TCA cycle and acetate metabolism			
CS	0.02 ± 0.01	0.25 ± 0.06	0.13 ± 0.05
Acn	0.16 ± 0.08[a]	0.31 ± 0.04[a]	0.21 ± 0.05[a]
ICDH	0.97 ± 0.05	0.59 ± 0.04	0.36 ± 0.05
SDH	0.09 ± 0.04[b]	0.14 ± 0.05[b]	0.15 ± 0.04[b]
FUM	0.011 ± 0.004[c]	0.021 ± 0.06[c]	0.013 ± 0.03[c]
MDH	0.006 ± 0.008	0.319 ± 0.06	0.19 ± 0.05
Icl	0.05 ± 0.009	0.33 ± 0.06	0.12 ± 0.03
MS	0.043 ± 0.008	0.29 ± 0.05	0.11 ± 0.04
Ack	0.81 ± 0.05	0.57 ± 0.06	0.13 ± 0.04
Acs	0.06 ± 0.005	0.10 ± 0.006	0.36 ± 0.07
Gluconeogenesis and others			
Fbp	0.041 ± 0.06	0.138 ± 0.05	0.088 ± 0.05
Pck	0.015 ± 0.08	0.15 ± 0.007	0.076 ± 0.05
MEZ (NAD^+-dependent)	0.02 ± 0.009	0.026 ± 0.04	0.028 ± 0.04
MEZ ($NADP^+$-dependent)	0.018 ± 0.006	0.075 ± 0.005	0.044 ± 0.008
LDH	0.04 ± 0.006	0.002 ± 0.001	0.002 ± 0.001

[a]Specific activity of total aconitase enzyme
[b]Specific activity of total succinate dehydrogenase enzyme
[c]Specific activity of total fumarase enzyme
EP: exponential phase; ES: early stationary phase; STA: stationary phase

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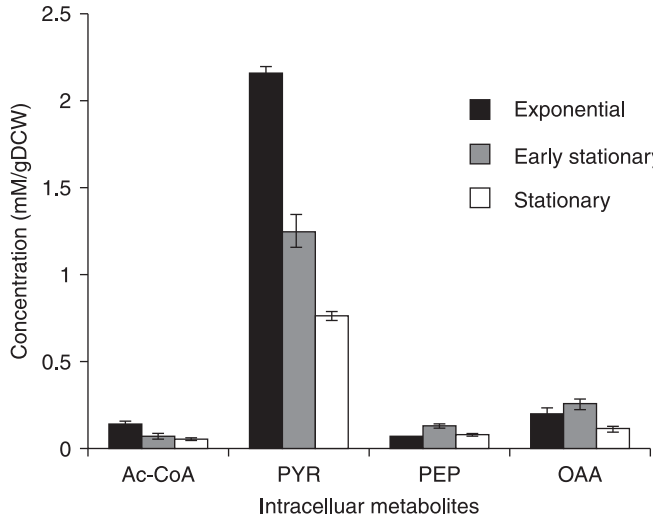

Figure 2.7 **Concentration of different intracellular metabolites. Intracellular concentrations of AcCoA, PYR, PEP, and OAA from samples collected at different phases of growth**

to maintain the required activities of the corresponding enzymes. With the exception of *fumA* and *fumC*, the results show high correlation between the total gene expression and the corresponding enzyme activities (Table 2.5). Whereas the gene expressions of *acnA*, *tktB*, and *talA* increased and those of *acnB*, *tktA*, and *talB* decreased as the culture proceeded toward stationary phases of growth, the gene expressions of *fumA* and *fumC* changed in a similar pattern (Figure 2.8). The higher expression of both *fumA* and *fumC* at the early stationary phase indicates that despite their similar physiological activities, *fum*A expression is modulated by extracellular acetate, while that of *fum*C is dependent on the growth phase and *rpoS* expression (Figures 2.6b) (Park and Gunsalus, 1995; Chen et al., 2001).

Regarding regulation of the TCA cycle, other global regulator such as ArcA must also be considered. The expression of the *arcA* gene is slightly changed at the early stationary and stationary phases of growth (Figure 2.6f). Although ArcA is known as a negative regulator of the TCA cycle under microaerobic conditions, some of the TCA cycle genes, such as *gltA*, *fumA*, *fum*C, and *acnA*, were up-regulated at the early stationary phase, while other genes, such as *icd*, *sodA*, and *acnB*, showed

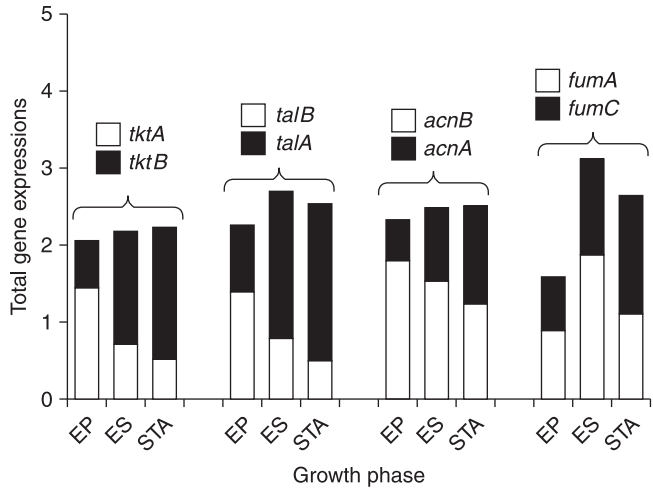

Figure 2.8 **Total and relative expression of iso-genes of *E. coli* metabolic pathways. Gene expression data were obtained by RT-PCR of samples collected at different phases of growth. EP: exponential phase; ES: early stationary phase; STA: stationary phase**

down-regulation (Figure 2.6f) (Alexeeva et al., 2003; Levanon et al., 2005). Therefore, the effect of ArcA was not dominant under aerobic growth conditions in the present case.

In summary, the above phenomenon demonstrates that the growth phase-dependent changes in *rpoS* expression are the most prominent of global regulators related to *E. coli* metabolism. Increased expression of the *rpoS* gene during the later phases of growth is coordinated with the changes in the expression of other regulators, with the *rpo*S effect at times overriding the effects of other regulators of vital metabolic pathway genes to cope with the changes in extracellular carbon sources and carbon starved conditions.

2.4 Reduction of by-product (acetate) formation

One of the main obstacles for producing the particular metabolite is the formation of by-products such as acetate in *E. coli* or ethanol in yeast.

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Rapid aerobic growth of *E. coli* on glucose as well as on gluconate, pyruvate, lactate, glucuronate, and serine but not on glycerol or fructose, and glucose excess conditions are characterized by the formation and excretion of acetate (Veit et al., 2007). This phenomenon is referred to as overflow metabolism or the bacterial Crabtree effect, and its mechanism has been investigated to reduce acetate formation (El-Mansi and Holms, 1989; Rinas et al., 1989; Luli and Strohl, 1990; Holms, 1996; Xu et al, 1999a,b; El-Mansi, 2004; Wolfe, 2005; DeMey et al., 2007; Veit et al., 2007).

It is commonly observed that *E. coli* excretes 10 to 30% of carbon flux from glucose to acetate in glucose-containing media, even when the culture is fully aerated. Acetate excretion is a major limitation in the high cell density culture to obtain high protein concentration (Klenan and Strohl, 1994; van de Walle and Shiloach, 1998). Under aerobic conditions, acetate is generated from pyruvate, either by oxidative decarboxylation by the pyruvate dehydrogenase complex (PDHc), followed by the conversion of acetyl-CoA (AcCoA) to acetate by phosphotransacetylase (Pta) and acetate kinase (Ack) with concomitant formation of ATP at Ack reaction (Hansen and Henning, 1966), or by decarboxylation to acetate directly by pyruvate oxidase (Pox) (Chang et al., 1994) (Figure 2.9). The latter is utilized usually during transition from exponential growth phase to stationary phase under the control of RpoS. After glucose depletion or at low concentration, acetate is assimilated by AcCoA sysnthetase (ACS) with concomitant conversion of ATP to AMP and pyrophosphate (Brown et al., 1977; Kumari et al., 1995), where *acs* is also under control of RpoS. The AcCoA thus formed is metabolized by both the TCA cycle and the glyoxylate pathway for energy generation and cell synthesis. Since acetate is produced mainly through the actions of Pta (encoded by *pta*) and Ack (encoded by *ackA*), genetic techniques have been applied to obtain *pta* mutant, which showed decreased acetate production (Bauer et al., 1990). However, *pta* gene knockout leads to high pyruvate production, which is also undesirable (Diaz-Ricci et al., 1991). It is shown that the Pta-Ack pathway dominates in the exponential growth phase, and the Pox pathway dominates for acetate production in the stationary phase, and that the former pathway is repressed under acidic conditions, whereas the Pox pathway is activated (Dittrich et al., 2005).

E. coli is used for the production of heterologous proteins (Swartz, 2001) since recombinant DNA technology has become available (Cohen et al., 1973). *E. coli* strains were also engineered for the production of chemicals and building blocks (Wendisch et al., 2006). As already

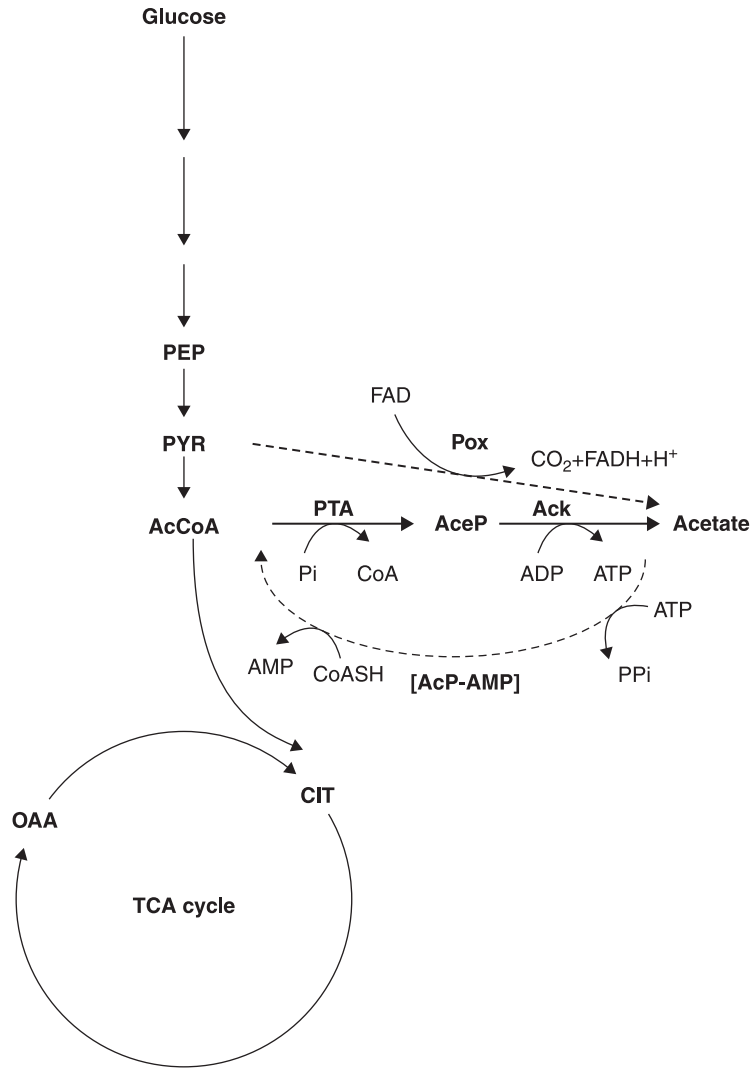

Figure 2.9 Acetate metabolism

mentioned, one of the problems is the acetate formation. Some attempt has, therefore, been made at recombinant protein production (Wong et al., 2008).

Aerobic acetate production is particularly significant at higher growth rates (El-Mansi and Holms, 1989; Majewski and Domach, 1990; Kayser et al., 2005; Wong et al., 2008), and it is a manifestation of the imbalance

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between glucose uptake and the demands for both biosynthesis and energy production (El-Mansi and Holms, 1989). The most common arguments are that the glucose uptake rate is improperly controlled and that the activity of the TCA cycle is limiting. One way to lower acetate production is to construct mutants with modified glucose uptake rates (Chou et al., 1994). Using this strategy, the TCA cycle can handle all the AcCoA produced by the glycolytic pathway, thus it can eliminate acetate formation. Acetate production may be also reduced by decreasing the glucose uptake rate by decreasing the glucose concentrations in the fermentor in the fed-batch cultivations (Akesson et al., 2001) or by decreasing the expression of *ptsG* that encodes the glucose-specific enzyme II (E II CB^{Glc}) of the phosphotransferase system (PTS) through expression of the *mlc* gene, which encodes Mlc that represses *ptsG* (Hosono et al., 1995; Cho et al., 2005), or by *ptsG* mutation (Flores et al., 2002). Alternatively, enhancement of the TCA cycle and the glyoxylate shunt also apparently reduces acetate production (Farmer and Liao, 1997). It has been reported that the constitutive expression of glyoxylate pathway genes may reduce acetate production by comparing *E. coli B* with *E. coli K* strain (Phue and Shiloach, 2004). The glyoxylate shunt contains two enzymes, isocitrate lyase (Icl) (encoded by *aceA*) and malate synthase (encoded by *aceB*), of which genes are located on the *aceBAK* operon, with *aceK* coding for ICDH kinase/phosphatase. The transcriptional regulation of this operon involves many factors, including IclR, FadR, Cra, ArcAB, and HimAB (Cronan and Laporte, 1999). The expression of *aceBAK* is induced during growth on either acetate or fatty acids, but induction is repressed in the presence of glucose, glycerol, or pyruvate. Mutation in *fadR* results in transcriptionally increased expression of *aceBAK*, even for such carbon sources (Gui et al., 1996) and affects the metabolism (Peng and Shimizu, 2006). Another way of reducing acetate production is to enhance anaplerosis via increasing PEP carboxylase (Ppc) and/or glyoxylate pathway enzyme levels (Farmer and Liao, 1997).

Acetate and other short-chain fatty acids inhibit cell growth and deteriorate metabolite production (Jensen and Carlsen, 1990), as they uncouple the trans-membrane protein potential and thus interfere with efficient energy metabolism. Acetic acid diffuses across the plasma membrane and the cytoplasma protein is released to form the acetate anion, resulting in a decrease of the trans-membrane protein potential (Axe and Bailey, 1995).

Note that carbon flux toward acetate production can be redirected to other less toxic metabolites, such as acetone (Bermejo et al.,

1998), acetoin (Aristidou et al., 1994), lactate (Chang et al., 1999a), polyhydroxybutyrate (PHB) (Chang et al., 1999b), or ethanol (Diaz-Ricci et al., 1991).

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