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MINI-REVIEW

Engineering of NADPH regenerators in *Escherichia coli* for enhanced biotransformation

Won-Heong Lee · Myoung-Dong Kim · Yong-Su Jin · Jin-Ho Seo

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Abstract Efficient regeneration of NADPH is one of the limiting factors that constrain the productivity of biotransformation processes. In order to increase the availability of NADPH for enhanced biotransformation by engineered *Escherichia coli*, modulation of the pentose phosphate pathway and amplification of the transhydrogenases system have been conventionally attempted as primary solutions. Recently, other approaches for stimulating NADPH regeneration during glycolysis, such as replacement of native glyceradehdye-3-phosphate dehydrogenase (GAPDH) with NADP-dependent GAPDH from *Clostridium acetobutylicum* and introduction of NADH kinase catalyzing direct phosphorylation of NADH to NADPH from *Saccharomyces cerevisiae*, were attempted and resulted in remarkable impacts on NADPH-dependent bioprocesses. This review summarizes several metabolic engineering approaches used for improving the NADPH regenerating capacity in engineered *E. coli* for whole-cell-based bioprocesses and discusses the key features and progress of those attempts.

Keywords Biotransformation process · Engineered *Escherichia coli* · NADPH regeneration · Pentose phosphate pathway · Transhydrogenase · NADP-dependent glyceradehdye-3-phosphate dehydrogenase · NAD(H) kinase

W.-H. Lee · J.-H. Seo (✉)
Department of Agricultural Biotechnology and Center for Food and Bioconvergence, Seoul National University, Seoul 151-921, Korea
e-mail: jhseo94@snu.ac.kr

W.-H. Lee · Y.-S. Jin
Department of Food Science and Human Nutrition and Institute for Genomic Biology, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA

M.-D. Kim
Department of Food Science and Biotechnology, Kangwon National University, Chuncheon 200-701, Korea

Introduction

Biotransformation processes hold many advantages over conventional chemical processes as biotransformation provides more benefits such as selectivity, safety, and cleanness (Drepper et al. 2006; Duetz et al. 2001). Enzymes catalyzing a single-step reaction or cells performing multistep reactions are essential parts of biotransformation because they not only exhibit higher substrate specificity and product selectivity, but also work well even under mild reaction condtions (Drepper et al. 2006; Duetz et al. 2001). Between the two types of biotransformation, the whole-cell-based biotransformation (with living microorganisms) may be more favorable than the enzyme-based biotransformation in terms of cost-saving and biocatalyst stability. However, the whole-cell-based biotransformation may be difficult to optimize because it is usually facilitated by numerous complicated reactions (or metabolic pathways) and requires specific cofactors (Duetz et al. 2001; van der Donk and Zhao 2003). The overall performance of the whole-cell biotransformation is often limited by cofactor regeneration because cells have intended not to sustain the biotransformation, but rather to grow better (Duetz et al. 2001; Park 2007). In particular, NADPH is one of the most important cofactors for producing a number of nutraceuticals and fine chemicals (Duetz et al. 2001; Park 2007; van der Donk and Zhao 2003). Because NADPH is an essential component for the anabolism (biosynthesis of cellular components) inside the cell (Holm et al. 2010; Sauer et al. 2004; van der Donk and Zhao 2003), NADPH regenerating systems should be complemented in order to enhance the productivity of the whole-cell biotransformation processes.

An engineered *Escherichia coli* has been used as a host for the NADPH-dependent production of many value-added materials. In those cases, endogenous enzymes involved in NADPH generation were engineered to improve productivity and product titers. Three pathways are known to play major roles in NADPH generation. These include the oxidative part of the pentose phosphate pathway (PPP), the tricarboxylic acid cycle (TCA), and the transhydrogenases system. They are reported to provide, respectively, 35–45, 20–25, and 30–45 % of NADPH generation during the aerobic batch cultivation of *E. coli* on glucose (Sauer et al. 2004). Therefore, many studies have focused on the modulation of the above pathways to increase intracellular NADPH availability. Along with the endogenous NADPH generating pathways, several heterologous NADPH generating enzymes have also been introduced to increase the intracellular NADPH level in engineered *E. coli*. The key enzymes and metabolic pathways, which have been engineered for efficient regeneration of NADPH in *E. coli*, were illustrated in Fig. 1.

This review introduces various approaches to enhance the product formation by engineered *E. coli* through metabolic engineering of NADPH generating pathways and discusses the key features of recent approaches for efficient regeneration of NADPH. Some examples of the NADPH-dependent biotransformation processes, in which the NADPH metabolic pathways were engineered, were illustrated in Figs. 2 and 3. In addition, we propose yet untested potential candidates for NADPH regeneration in the whole-cell biotransformation processes.

Pentose phosphate pathway

As shown in Fig. 1, glucose-6-phosphate dehydrogenase (G6PDH, EC 1.1.1.49) and 6-phosphogluconate dehydrogenase (Gnd, EC 1.1.1.44) are involved in NADPH generation in the oxidative part of the PPP. Because overexpression of G6PDH can increase the flux for the Gnd catalyzing reaction, G6PDH was more commonly overexpressed in the NADPH-dependent biotransformation processes, as observed in a previous report where overexpression of G6PDH was three times more effective than Gnd overexpression for increasing the NADPH level in *E. coli* (Lim et al. 2002). As shown in Fig. 3a, the PhbB catalyzing reaction requires NADPH in the synthesis of poly-3-hydroxybutyrate (PHB). Consequently, the G6PDH overexpressing strain produced more PHB than the Gnd overexpressing strain (Lim et al. 2002). Overexpression of G6PDH was also effective for the Baeyer–Villiger (BV) oxidation of cyclohexanone that requires NADPH as a cofactor (Fig. 2a). The overexpression of G6PDH showed a 39 % enhancement of ε-caprolactone production compared with the control (Lee et al. 2007).

Instead of overexpressing the enzymes in the PPP, redirection of metabolic fluxes from glycolysis to the PPP has also been attempted to increase intracellular NADPH levels. Deletion of the *pgi* gene encoding phosphoglucose isomerase (Pgi, EC 5.3.1.9), the first enzyme shunting glucose-6-phosphate into glycolysis, may be a representative example. Deletion of the *pgi* gene increased the NADPH-dependent production of leucocyanidin by 4-fold and catechin by 2-fold from dihydroquercetin (Fig. 2b) in engineered *E. coli* (Chemler et al. 2010). It was also reported that inactivation of Pgi increased the biosynthesis of thymidine by 4-fold in engineered *E. coli* (Lee et al. 2010) because NADPH is used not only for conversion of UDP to dUDP but also for the recycling of tetrahydrofolate to dihydrofolate, a co-substrate for dTMP in the thymidine biosynthesis (Fig. 3b).

Because fructose-6-phosphate, one of the final products from the PPP, can return to the PPP by the reversible reaction of Pgi, the phosphofructokinase genes (*pfkA* and *pfkB*) were also engineered to increase the metabolic flux to the PPP instead of the *pgi* gene deletion. In addition, the genes that are involved in the branches of glycolysis such as the fermentative pathway (or respiratory pathway) were also manipulated in order to maximize the carbon flux to the PPP. In those studies, the product yield per glucose consumption (Y_{RPG}, a molar ratio of a product to glucose consumed) was used as a standard parameter to evaluate the efficiency of NADPH regeneration from glucose with assumptions that the conversion of a substrate to a product is proportional to the amount of glucose consumed for NADPH generation. For instance, deletion of both *pfkA* and *pfkB* genes enhanced Y_{RPG} of methyl-3-hydroxybutyrate (MHB) production from methyl acetoacetate (MAA) by 44 % (Fig. 2c) compared with the *pgi* deletion case (Siedler et al. 2011). Inactivation of the three genes in the respiratory pathway (*ndh*, encoding NADH dehydrogenase) and in the fermentative pathway (*adhE*, encoding acetaldehyde dehydrogenase and *ldh*, encoding lactate dehydrogenase) resulted in a 29 % improvement in Y_{RPG} of propanol production (Fig. 2d) compared with the *pgi* deletion (Fasan et al. 2011).

As another way to amplify the PPP, a heterologous enzyme glucose dehydrogenase (GDH, EC 1.1.1.47) has been introduced into *E. coli*. GDH was identified in various Gram-positive bacteria such as *Bacillus subtilis* and *Bacillus megaterium* and functionally expressed in *E. coli* (Park et al. 2010; Zhang et al. 2011). As GDH generates NADPH by the oxidation of glucose to glucono-1,5-lactone that can be further converted to 6-phosphogluconate in the oxidative part of the PPP (Fig. 1), overexpression of GDH was expected to exert a similar effect like overexpression of G6PDH for NADPH-dependent oxidative (or reductive) biotransformation processes. Figure 2e–g shows the examples of NADPH-dependent reactions where GDH was introduced for the NADPH regeneration. Overexpression of GDH from *B. subtilis* enhanced asymmetric oxidation of

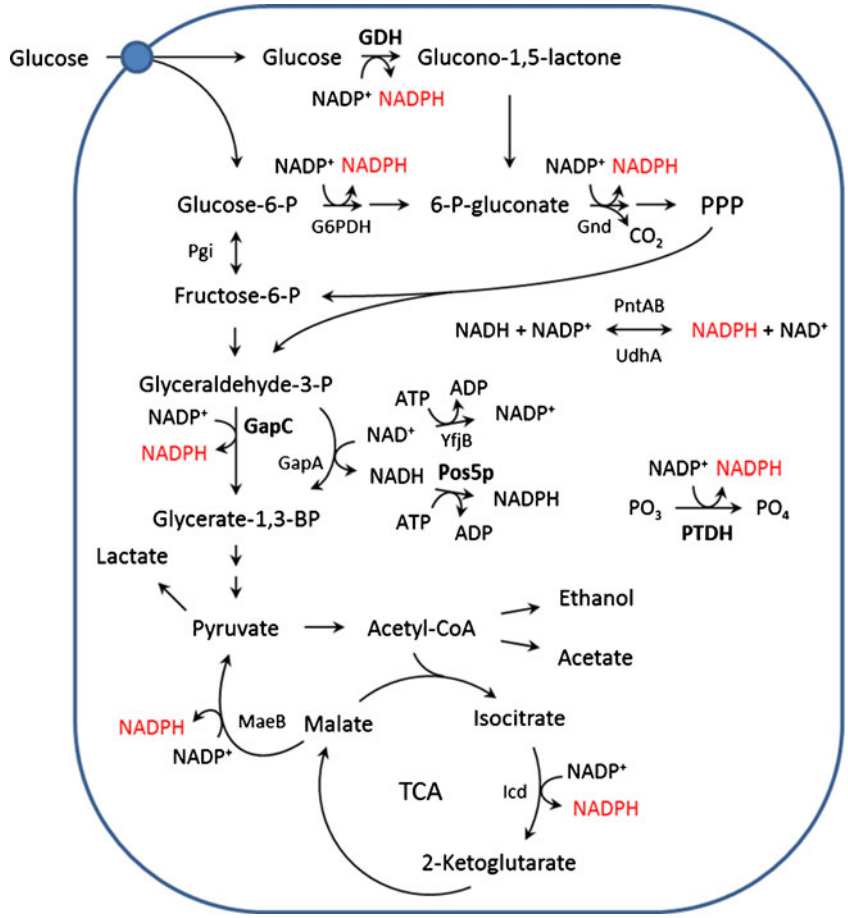

Fig. 1 The metabolic pathways and enzymes used for NADPH regeneration in NADPH-dependent biotransformation processes using engineered *E. coli*. The heterologous enzymes are typed in *bold*. The names of enzymes are abbreviated as follows: G6PDH, glucose-6-phosphate dehydrogenase; Gnd, 6-phosphogluconate dehydrogenase; Pgi, phosphoglucose isomerase; Icd, isocitrate dehydrogenase; MaeB, $NADP^+$-dependent malic enzyme; PntAB, membrane-bound transhydrogenase; UdhA, soluble transhydrogenase; GapA, native NAD-dependent glyceraldehyde-3-phosphate dehydrogenase (GAPDH); GapC, NADP-dependent GAPDH from *C. acetobutylicum*; YfjB, native NAD kinase; Pos5p, NADH kinase from *S. cerevisiae*; GDH, glucose dehydrogenase from *B. subtilis* (or *B. megaterium*); PTDH, engineered phosphite dehydrogenase from *P. stutzeri*

p-chlorothioanisole by 4-fold in recombinant *E. coli* (Zhang et al. 2011). Overexpression of GDH from *B. megaterium* was also reported to be effective on asymmetric reduction of ethyl-4-chloro-3-oxobutanoate (COBE) to ethyl-(*R*)-4-chloro-3-hydroxybutanoate (CHBE) (Xu et al. 2007). However, GDH was expressed in the cytosol and did not utilize glucose-6-phosphate but rather glucose as a substrate, which indicates that efficient transport of external glucose should be considered in order to facilitate a biotransformation rate. Consequently, co-overexpression of a glucose facilitator (from *Zymomonas mobilis*) with GDH was performed in the two-phase biotransformation system (aqueous–organic) where glucose transportation was severely inhibited by the organic phase. As a result, a 6.7-fold improvement of the production of α-pinene oxide was obtained (Schewe et al. 2008).

The modulation of the PPP has been attempted as a primary approach for increasing NADPH availability in biotransformation processes. However, this approach may cause a reduction of target product formation if a substrate is used for regenerating NADPH since one carbon molecule would be lost via the decarboxylation step of 6-phosphogluconate (Gnd catalyzing reaction). For instance, NADPH-dependent GDP-L-fucose biosynthesis from glucose (Fig. 3c) was reduced by overexpression of G6PDH under the glucose-limiting condition even though the G6PDH overexpressing strain showed higher intracellular NADPH level than the control strain (Lee et al. 2011). Optimization of the glucose feeding strategy improved GDP-L-fucose by 21 % with G6PDH overexpression (Lee et al. 2011), which suggests that sufficient supplementation of an energy source should be taken into account when the PPP is modulated in the NADPH-dependent biotransformation.

Tricarboxylic acid cycle and anaplerotic pathway

As shown in Fig. 1, isocitrate dehydrogenase (Icd, EC 1.1.1.42) is involved in NADPH generation in the TCA. Icd is known to be capable of supplying substantial amounts of NADPH in *E. coli* (Sauer et al. 2004), which was confirmed in a previous report where the inactivation of the TCA reduced Y_{RPG} of xylitol production by 32 % in NADPH-dependent xylitol production (Fig. 2h) (Chin et al. 2008). In addition, NADPH is known to be generated by a malic enzyme (MaeB, $NADP^+$-dependent malate dehydrogenase, EC 1.1.140) in the anaplerotic pathway (Wang et al. 2011). Therefore, overexpression of the *maeB* gene as

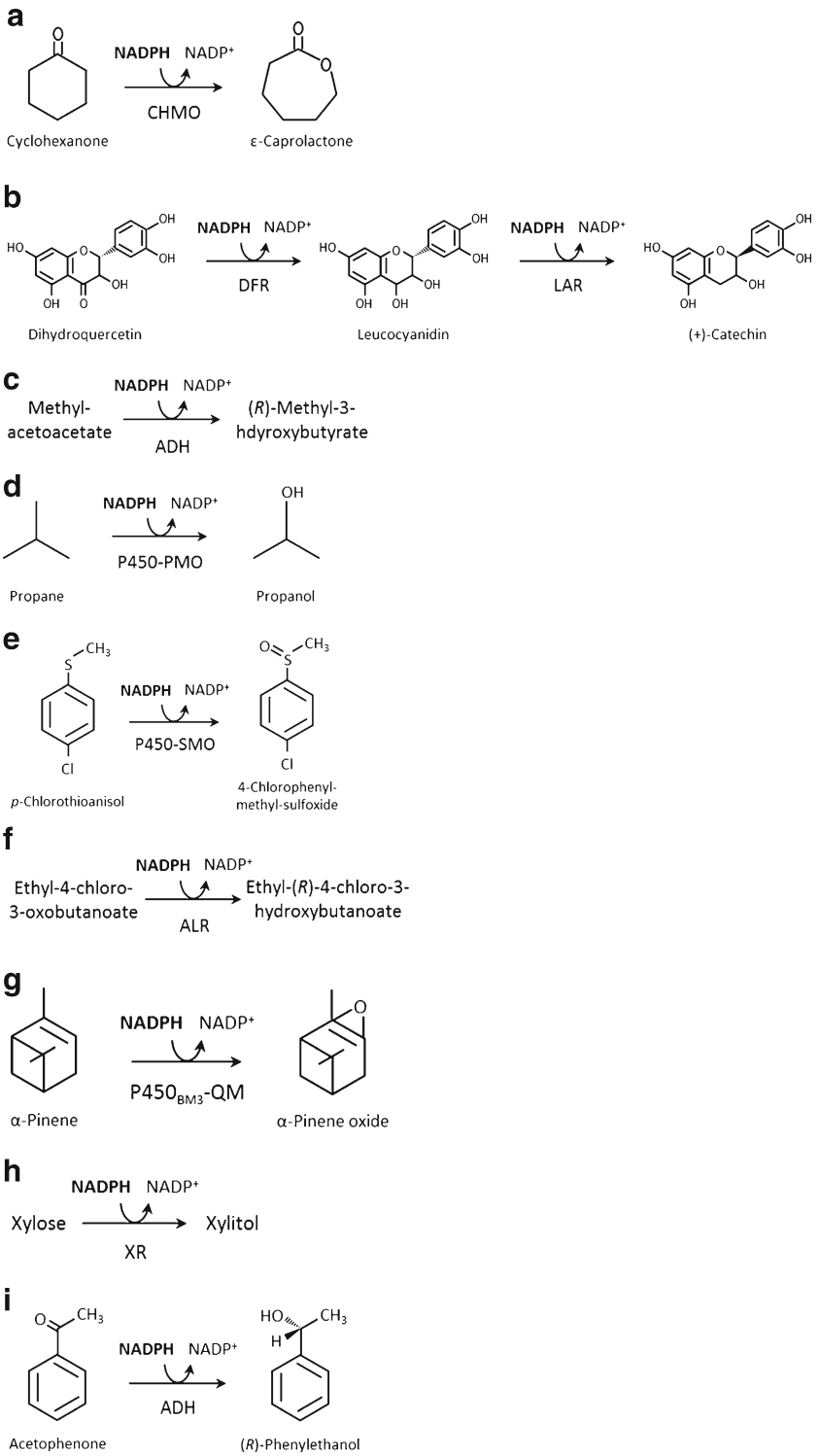

Fig. 2 Example of the NADPH-dependent single-step bioconversion processes and the names of enzymes involved in the bioconversions. **a** Production of ε-caprolactone from cyclohexanone; CHMO, cyclohexanone monooxygenase from *Acinetobacter calcoaceticus*. **b** Production of (+)-catechin and leucocyanidin from dihydroquercetin; DFR, dihydroflavonoid-4-reductase from *Anthurium andraeanum*; LAR, leucoanthocyanidin reductase from *Desmodium uncinatum*. **c** Production of methyl-3-hydroxybutyrate (MHB) from methyl acetoacetate (MAA); ADH, alcohol dehydrogenase from *Lactobacillus brevis*. **d** Production of propanol from propane; P450-PMO, variant of engineered cytochrome P450 monooxygenase from *B. megaterium*. **e** Production of 4-chlorophenyl-methy-sulfoxide, (*S*)-sulfoxide, from *p*-cholorothioanisole; P450-SMO, cytochrome P450 monooxygenase from *Rhodococcus* sp. **f** Production of ethyl-4-chloro-3-hydroxybutanoate (CHBE) from ethyl-4-chloro-3-oxobutanoate (COBE); ALR NADPH–dependent aldehyde reductase from S*porobolomyces salmonicolor*. **g** Production of α-pinene oxide from α-pinene; $P450_{BM3}$-QM, quintuple mutant of engineered cytochrome P450 monooxygenase from *B. megaterium*. **h** Production of xylitol from xylose; XR, xylose reductase from *Candida boidinii*. **i** Production of phenylethanol from acetophenone; ADH, alcohol dehydrogenase from *Lactobacillus kefir*

well as *icd* gene was tested to improve GDP-L-fucose biosynthesis in engineered *E. coli*. Although overexpression of Icd resulted in 30 % higher GDP-L-fucose production compared with the control (without overexpression of the NADPH regenerating enzyme), Icd overexpression did not show better effects on biosynthesis of GDP-L-fucose than G6PDH overexpression (Lee et al. 2011). What is worse, overexpression of MaeB resulted in a 24 % reduction of GDP-L-fucose production compared with the control (Lee et al. 2011), which might be due to fact that the anaplerotic pathway is activated at a certain metabolic state such as synthesis of pyruvate from dicarboxylic acids under anaerobic conditions (Kwon et al. 2007). These results suggest that both Icd and MaeB may not

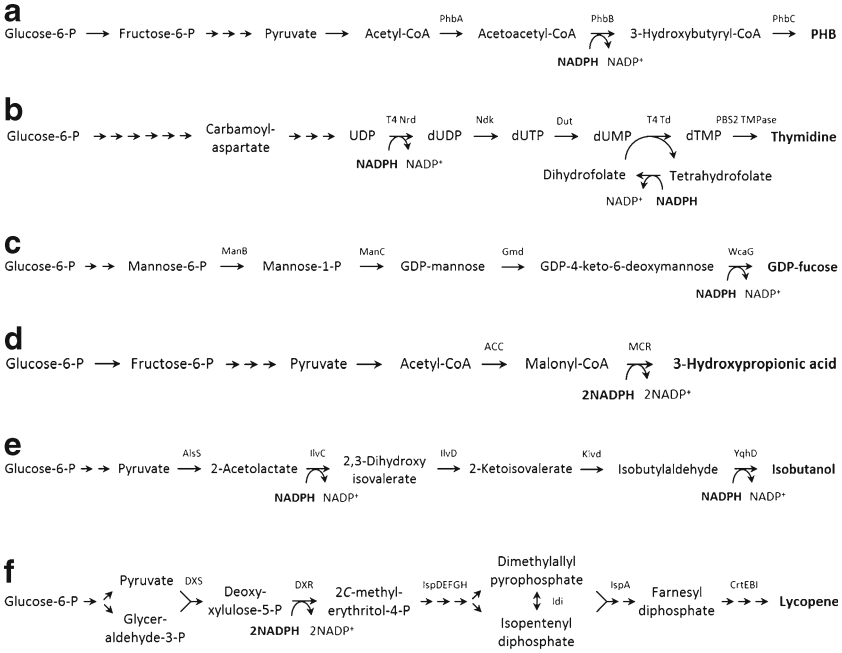

Fig. 3 Example of the NADPH-dependent multistep biosynthetic processes and the names of enzymes involved in the biosynthetic pathways. **a** Poly-3-hydroxybutyrate (PHB) biosynthesis; PhbA, β-ketothiolase from *Alcaligenes eutrophus*; PhbB, acetoacetyl-CoA reductase from *A. eutrophus*; PhbC, PHB synthase from *A. eutrophus*. **b** Thymidine biosynthesis; T4 Nrd, nucleotide diphosphate reductase from T4 phage; Ndk, nucleotide diphosphate kinase in *E. coli*; Dut, deoxyribonucleotide triphosphatase in *E. coli*; T4 Td, thymidylate synthase from T4 phage; PBS2 TMPase, thymidine monophosphate phosphohydrolase from T4 phage. **c** GDP-L-fucose biosynthesis; ManB, phosphomannomutase in *E. coli*; ManC, mannose-1-phosphate guanylyltransferase in *E. coli*; Gmd, GDP-D-mannose-4,6-dehydratase in *E. coli*; WcaG, GDP-L-fucose synthase in *E. coli*. **d** 3-Hydroxypropionic acid (3-HP) biosynthesis; ACC, acetyl-CoA carboxylase in *E. coli*; MCR, malonyl-CoA reductase from *Chloroflexus aurantiacus*. **e** Isobutanol biosynthesis; AlsS, acetolactate synthase from *B. subtilis*; IlvC, ketol-acid reductoisomerase in *E. coli*; IlvD, dihydroxy-acid dehydratase in *E. coli*; Kivd, 2-ketoisovalerate decarboxylase from *Lactococcus lactis*; YqhD, alcohol dehydrogenase in *E. coli*. **f** Lycopene biosynthesis; DXS, 1-deoxy-D-xylulose-5-phosphate synthase in *E. coli*; DXR, 2*C*-methyl-D-erythritol-4-phosphate synthase in *E. coli*; IspD, 4-diphosphocytidyl-2*C*-methyl-D-erythritol-4-phosphate synthase in *E. coli*; IspE, 4-diphosphocytidyl-2*C*-methyl-D-erythritol kinase in *E. coli*; IspF, 2*C*-methyl-D-erythritol-2,4-cyclodiphosphate synthase in *E. coli*; IspG, 1-hydroxy-2-methyl-2-(*E*)-butenyl-4-diphosphate synthase in *E. coli*; IspH, 1-hydroxy-2-methyl-2-(*E*)-butenyl-4-diphosphate reductase in *E. coli*; Idi, isopentenyl diphosphate isomerase in *E. coli*; IspA, farnesyl diphosphate synthase in *E. coli*; CrtE, geranylgeranyl diphosphate synthase from *Erwinia herbicola*; CrtB, phytoene synthase from *E. herbicola*; CrtI, phytoene desaturase from *E. herbicola*

be better options for NADPH regeneration in biotransformation than G6PDH.

Transhydrogenases system

Along with the enzymes in the oxidative part of the PPP, a transhydrogenase system has been often used in NADPH-dependent biotransformation processes because NADPH can be generated by transhydrogenase enzymes in *E. coli* (Sauer et al. 2004). There are two types of transhydrogenases in *E. coli* including membrane-bound transhydrogenase (PntAB, EC 1.6.1.2) and soluble transhydrogenase (UdhA, EC 1.6.1.1) (Fig. 1). While the energy-dependent, membrane-bound PntAB catalyzes the transfer of a hydrogen ion from NADH to $NADP^+$ (production of NADPH) under low levels of NADPH, the energy-independent, cytosolic UdhA transfers a hydrogen ion from NADPH to NAD^+ (production of NADH) under high concentrations of NADPH (Boonstra et al. 2000; Canonaco et al. 2001; Holm et al. 2010; Sauer et al. 2004). Even though UdhA is known to control cellular redox balance when the NADPH level is high (Canonaco et al. 2001; Holm et al. 2010) and convert NADPH and NAD^+ to $NADP^+$ and NADH (Boonstra et al. 1999; Cao et al. 2011), overexpression of UdhA was reported to be effective for increasing NADPH availability in *E. coli*. For example, overexpression of UdhA improved NADPH-dependent PHB production by 82 % and specific PHB content by 35 % in engineered *E. coli* (Sanchez et al. 2006). In another study, overexpression of UdhA also led to a 2-fold increase in NADPH-dependent thymidine production in engineered *E. coli* (Lee et al. 2010). Considering the altered intracellular levels of pyridine nucleotides and the higher expression levels of $NADP^+$-dependent dehydrogenases (e.g., G6PDH and Gnd) by UdhA overexpression (Lee et al. 2010; Sanchez et al. 2006), it is evident that UdhA is involved in regeneration of NADPH indirectly when overexpressed in *E. coli*.

Overexpression of PntAB has also been effective for enhancing the productivity of NADPH-dependent bioprocesses in *E. coli*. PntAB was necessary for the NADPH-dependent production of xylitol from xylose in engineered *E. coli* because deletion of the *pntAB* genes reduced Y_{RPG} of xylitol production by 55 % (Akinterinwa and Cirino 2011). Overexpression of PntAB enhanced the conversion of acetophenone to (*R*)-phenylethanol by 3.5-fold (Fig. 2i) (Weckbecker and Hummel 2004). As the biosynthesis of 3-hydroxypropionic acid (3-HP) requires 2 mol of NADPH at the end of 3-HP biosynthesis (Fig. 3d), PntAB was overexpressed in *E. coli* and resulted in a 34 % improvement in the final concentration of 3-HP compared with the control (Rathnasingh et al. 2012). PntAB was functionally expressed and significantly improved the NADPH-dependent L-lysine production even in *Corynebacterium glutamicum* (Kabus et al. 2007).

Recently, overexpression of PntAB exerted a positive effect on NADPH-dependent isobutanol biosynthesis under complete anaerobic conditions (Bastian et al. 2011). As shown in Fig. 3e, the conversion of glucose to isobutanol requires 2 mol of NADPH in the YqhD catalyzing reaction as well as the IlvC catalyzing reaction. Under anaerobic conditions, however, it was difficult to supply sufficient amounts of NADPH via the PPP for sustaining isobutanol synthesis because the oxidative part of the PPP is not functional enough under anaerobic conditions. Consequently, 1.5 g/L of isobutanol was produced in engineered *E. coli* under anaerobic conditions (Bastian et al. 2011), which was consistent with a previous report which already revealed that the carbon flux to the oxidative PPP in the anaerobic glucose culture was more than two times lower than the aerobic glucose culture of *E. coli* (Sauer et al. 1999). In order to solve a cofactor imbalance problem for isobutanol production, they overexpressed PntAB and obtained a 6.5-fold enhancement of isobutanol production under anaerobic conditions compared with the control (Bastian et al. 2011). A similar approach (modulation of PntAB expression through engineered promoters and integration rather than PntAB overexpression using a multicopy plasmid) led to an improvement of isobutanol production in *E. coli*, resulting in 20 % increase of isobutanol titer under aerobic and anaerobic conditions (Shi et al. 2013). However, it may be controversial to conclude that PntAB works well regardless of aeration because overexpression of PntAB did not work at all in NADPH-dependent 3-HP biosynthesis under the anaerobic condition (Rathnasingh et al. 2012).

It is interesting to note that 58 % more isobutanol was produced by changing the cofactor specificity of NADPH-dependent enzymes (from NADPH-dependent to NADH-dependent) than PntAB overexpression (Bastian et al. 2011). This result indicated that glycolysis can provide sufficient amounts of NADH necessary for isobutanol production under the anaerobic condition and that a cell can regenerate NADH more efficiently than NADPH.

NADPH regeneration in glycolysis by introduction of NADP-dependent glyceraldehyde-3-phosphate dehydrogenase (GapC) and NADH kinase (Pos5p)

Compared with NADPH, NADH can be easily generated through several pathways regardless of the presence of oxygen. In particular, an excess amount of NADH can be generated during glycolysis when the cell is growing on glucose. The main source for NADH supply during glycolysis is glyceraldehyde-3-phosphate (GAP) dehydrogenase (GAPDH, EC 1.2.1.12). As glycolysis can provide a sufficient amount of NADH for sustaining NADH-dependent reactions even under the anaerobic conditions (Bastian et al. 2011), some researchers attempted to generate NADPH instead of NADH during glycolysis by modifying native GAPDH (Martínez et al. 2008). They replaced the native NAD-dependent native *gapA* gene with a heterologous *gapC* gene encoding NADP-dependent GAPDH (EC 1.2.1.13) from *Clostridium acetobutylicum*. Since 16 mol of NADPH is required for the biosynthesis of 1 mol of lycopene (Fig. 3f) (Alper et al. 2005), they employed the GAPDH replaced strain for enhancing lycopene production. They also used the mutant strain for the BV oxidation of cyclohexanone requiring NADPH (Fig. 2a). With the aid of GapC, NADPH-dependent production of lycopene and ε-caprolactone was enhanced by 150 and 95 %, respectively (Martínez et al. 2008). Especially, overexpression of GapC significantly reduced the carbon flux to the PPP (80 % decrease), suggesting that the glycolytic pathway can be feasible for regenerating NADPH by changing the cofactor specificity of GAPDH.

Another approach for producing NADPH during glycolysis was direct phosphorylation of NADH to NADPH in *E. coli*. Although *E. coli* has only NAD kinase (YfjB, EC 2.7.1.23) that is known to have extremely low specificity to NADH than NAD^+ (Kawai et al. 2001), several attempts were made to increase the NADPH level indirectly by overexpression of native YfjB. Interestingly, overexpression of YfjB could increase the product yield of PHB by 76 % (Li et al. 2009). Overexpression of YfjB also improved NADPH-dependent thymidine production by 25 % (Lee et al. 2009, 2010). These observations suggested that an oversupply of $NADP^+$ by overexpression of NAD kinase disturbs the intracellular redox balance of pyridine nucleotides, which enforces a change in the expression level of $NADP^+$-consuming enzymes (Lee et al. 2009, 2010; Li et al. 2009). However, modulation of YfjB expression alone did not change NADPH availability when NADPH-dependent isobutanol production was coupled with YfjB expression in

engineered *E. coli* (5 and 3 % improvement under aerobic and anaerobic conditions, respectively) (Shi et al. 2013). These results suggest that more research should be performed to draw a clear conclusion about the effect of NAD kinase overexpression on NADPH availability in *E. coli* as numerous unknown factors are involved in redox balance between NAD^+ and NADPH.

It is interesting to note that combinatorial modulation of expression levels of both *yfjB* and *pntAB* genes led to 28 and 80 % improvement of isobutanol production in *E. coli* under aerobic condition and anaerobic condition, respectively, compared with the control (Shi et al. 2013). Combined modulation of YfjB with PntAB also showed 6 and 50 % enhanced isobutanol production as compared to PntAB modulation alone under aerobic and anaerobic conditions, respectively. Considering that the combined reaction catalyzed by YfjB (Reaction 1) and PntAB (Reaction 2) is the same as the NADH kinase catalyzing reaction (Reaction 3), the above results indicate that additional modification of $NADP^+$-related enzymes such as PntAB may be required to obtain the desirable effect of NAD kinase overexpression (direct phosphorylation of NADH to NADPH during glycolysis).

$NAD^+ + ATP \rightarrow NADP^+$ (Reaction 1)
$NADP^+ + NADH \rightarrow NADPH + NAD^+$ (Reaction 2)
$NADH + ATP \rightarrow NADPH$ (Reaction 3)

Meanwhile, *Saccharomyces cerevisiae* has three native NAD(H) kinases including Utr1p, Yef1p, and Pos5p (Outten and Culotta 2003; Strand et al. 2003). Pos5p is localized in the mitochondria and exhibits much higher affinity toward NADH than NAD^+ (Outten and Culotta 2003; Strand et al. 2003). Therefore, expression of heterologous Pos5p (truncated protein lacking of mitochondrial targeting sequence) was attempted to verify whether direct phosphorylation of NADH could enhance the productivity of NADPH-dependent biotransformation processes in *E. coli*. Pos5p was functionally expressed in *E. coli* and led to a 51 % enhancement of GDP-L-fucose biosynthesis (Lee et al. 2013). Expression of Pos5p also enhanced NADPH-dependent BV oxidation of cyclohexanone by 96 % in *E. coli* (Lee et al. 2013). In this report, overexpression of Pos5p showed even better results in terms of final product concentration and productivity when compared with the case of G6PDH overexpression, which suggests that more carbon molecules could be saved by NADH kinase overexpression compared with G6PDH overexpression. Also, minimized accumulation of acetate was observed in both cases of GDP-L-fucose production and ε-caprolactone production because acetate formation is known to be associated with the perturbed redox balance of pyridine nucleotides such as substantial accumulation of NADH (De Mey et al. 2007; Holm et al. 2010). These results also indicate that direct phosphorylation of NADH can be considered as a feasible method for NADPH regeneration in biotransformation processes.

Probably, heterologous expression of NADH kinase may be a relatively simpler strategy to regenerate NADPH during glycolysis because additional manipulation of $NADP^+$-consuming enzymes (e.g., PntAB) is not necessary compared with the native NAD kinase overexpression. However, NADH kinase consumes 1 mol of ATP for producing 1 mol of NADPH, possibly leading to reduced product yield because of dissipation of a carbon source for ATP formation under the anaerobic conditions where ATP production is much less than aerobic conditions. In addition, another redox imbalance problem might occur when NADH kinase is used for the NADPH regenerating system because the GAPDH catalyzing reaction (Reaction 5) is the main source for NADH during glycolysis under anaerobic conditions. Comparing the NADH kinase reaction (Reaction 3) with other NADPH regenerating reactions (e.g., PntAB or NADP-dependent GAPDH catalyzing reaction, Reaction 2 or 4) stoichiometrically, the net reaction with NADH kinase (Reaction 7) would result in an oversupply of $NADP^+$ as compared to other reactions (Reactions 8 and 9). This oversupply of $NADP^+$ might require enzymes to return $NADP^+$ to NAD^+. The net reactions of NADPH-dependent reactions (e.g., xylitol production, Reaction 6) coupled with NADPH-regenerating reactions are summarized as follows:

$NADP^+ \rightarrow NADPH$ (Reaction 4)
$NAD^+ \rightarrow NADH$ (Reaction 5)
$Xylose + NADPH \rightarrow Xylitol + NADP^+$ (Reaction 6)
$NAD^+ + ATP + Xylose \rightarrow Xylitol + ADP + NADP^+$ (Reaction 7: sum of 3, 5, and 6)
$Xylose \rightarrow Xylitol$ (Reaction 8: sum of 2, 5, and 6)
$Xylose \rightarrow Xylitol$ (Reaction 9: sum of 4 and 6)

Progress from the recent studies is summarized in Tables 1 (single-step bioconversion) and 2 (multistep biosynthesis).

Other heterologous enzyme: engineered phosphite dehydrogenase (PTDH)

Phosphite dehydrogenase (PTDH, EC 1.20.1.1) from *Pseudomonas stutzeri* is a newly developed NADPH regenerating enzyme for enzymatic conversion of prochiral (or racemic) compounds to pure chiral compounds that can be a ready-to-use material for the synthesis of pharmaceuticals and fine chemicals (Johannes et al. 2006; Rioz-Martínez et al. 2011). Since the wild-type PTDH could not accept $NADP^+$ as a substrate, it was engineered to have higher affinity to $NADP^+$ through several site-directed mutageneses (Johannes et al. 2006; Woodyer et al. 2005). The engineered PTDH improved enzymatic synthesis of (*R*)-

Table 1 Progress of NADPH regeneration in the single-step bioconversion processes with engineered *E. coli*

Approaches	Substrates	Products	Carbon sources for NADPH	Culture conditions	Genetic manipulations	Key features		References
						Control	Experimental	
Modulation of the pentose phosphate pathway	Cyclohexanone	ε-Caprolactone	Glucose	Aerobic pH-stat fed-batch culture	G6PDH overexpression	Final ε-caprolactone —11.0 g/L Productivity—0.82 g/L h Y_{RPG}[a]—0.22 mol ε-caprolactone/mol glucose	Final ε-caprolactone —15.3 g/L Productivity—0.94 g/L h Y_{RPG}—0.27 mol ε-caprolactone/mol glucose	Lee et al. (2007)
	Dihydroquercetin	(+)-Catechin	Glucose	Aerobic batch culture	*pgi* gene deletion	Final (+)-catechin —18.6 mg/L	Final (+)-catechin —35.3 mg/L	Chemler et al. (2010)
		Leucocyanidin			*pgi* gene deletion	Final leucocyanidin —197.8 mg/L	Final leucocyanidin —756.0 mg/L	
	Propane	Propanol	Glucose	Aerobic bioconversion with resting cell	*pgi* gene deletion	Y_{RPG}—0.52 mol propanol/mol glucose	Y_{RPG}—1.33 mol propanol/mol glucose	Fasan et al. (2010)
					ndh, *ahdE*, and *ldh* genes triple deletion		Y_{RPG}—1.71 mol propanol/mol glucose	
	MAA	MHB	Glucose	Aerobic bioconversion with resting cell	*pgi* gene deletion	Y_{RPG}—2.44 mol MBH/mol glucose	Y_{RPG}—3.78 mol MBH/mol glucose	Siedler et al. (2011)
					pfkA and *pfkB* genes double deletion		Y_{RPG}—5.46 mol MBH/mol glucose	
	COBE	(*R*)-CHBE	Glucose	Aerobic bioconversion with resting cell	Overexpression of GDH from *B. megaterium*	COBE conversion yield—27.3 %	COBE conversion yield—100 %	Xu et al. (2007)
	α-Pinene	α-Pinene oxide	Glucose	Aerobic bioconversion with resting cell	Overexpression of GDH from *B. megaterium* and Glf from *Z. mobilis*	Specific α-pinene oxide production—3 mg/g cell	Specific α-pinene oxide production —20 mg/g cell	Schewe et al. (2008)
	p-Chlorothioanisole	(*S*)-Sulfoxide	Glucose	Aerobic bioconversion with resting cell	Overexpression of GDH from *B. subtilis*	Final (*S*)-sulfoxide —0.5 mM	Final (*S*)-sulfoxide —2 mM	Zhang et al. (2011)
Modulation of the transhydrogenases system	Acetophenone	(*R*)-Phenylethanol	Glucose	Aerobic bioconversion with resting cell	PntAB overexpression	Final (*R*)-phenylethanol —1.9 mM	Final (*R*)-phenylethanol —6.6 mM	Weckbecker and Hummel (2004)
Generation of NADPH during glycolysis	Cyclohexanone	ε-Caprolactone	Glucose	Aerobic batch culture	Replacement of native GapA with GapC from *C. acetobutylicum*	Productivity—0.28 g/L h Y_{RPG}—1.72 mol ε-caprolactone/mol glucose	Productivity—0.56 g/L h Y_{RPG}—2.97 mol ε-caprolactone/mol glucose	Mártinez et al. (2008)
	Cyclohexanone	ε-Caprolactone	Glucose	Aerobic pH-stat fed-batch culture	Overexpression of Pos5p from *S. cerevisiae*	Final ε-caprolactone —11.0 g/L Productivity —0.82 g/L h Y_{RPG}—0.22 mol ε-caprolactone/mol glucose	Final ε-caprolactone —21.6 g/L Productivity—1.60 g/L h Y_{RPG}—0.41 mol ε-caprolactone/mol glucose	Lee et al. (2013)

[a] Y_{RPG}: the product yield per consumed glucose (mole of product/mole of consumed glucose)

Table 2 Progress of NADPH regeneration in the multistep biosynthetic processes with engineered *E. coli*

Approaches	Substrates	Products	Carbon sources for NADPH	Culture conditions	Genetic manipulations	Key features		References
						Control	Experimental	
Modulation of the pentose phosphate pathway	Glucose	PHB	Glucose	Aerobic batch culture	Gnd overexpression G6PDH overexpression	Specific PHB content—0.13 g PHB/g cell	Specific PHB content—0.18 g PHB/g cell Specific PHB content—0.41 g PHB/g cell	Lim et al. (2002)
	Glucose	GDP-L-fucose	Glucose	Aerobic glucose limited fed-batch culture	G6PDH overexpression	Final GDP-L-fucose—170.3 mg/L Productivity—6.7 mg/L h Yield—1.2 mg GDP-L-fucose/g glucose	Final GDP-L-fucose—141.8 mg/L Productivity—5.8 mg/L h Yield—1.1 mg GDP-L-fucose/g glucose	Lee et al. (2011)
				Aerobic pH-stat fed-batch culture		Final GDP-L-fucose—193.6 mg/L Productivity—10.2 mg/L h Yield—1.0 mg GDP-L-fucose/g glucose	Final GDP-L-fucose—235.2 mg/L Productivity—12.7 mg/L h Yield—1.3 mg GDP-L-fucose/g glucose	
	Glucose	Thymidine	Glucose	Aerobic pH-stat fed-batch culture	*pgi* gene deletion	Final thymidine—0.21 g/L Productivity—3.4 mg/L h Yield—1.4 mg thymidine/g glucose	Final thymidine—1.02 g/L Productivity—15.8 mg/L h Yield—6.1 mg thymidine/g glucose	Lee et al. (2010)
Modulation of the transhydrogenases system	Glucose	PHB	Glucose	Aerobic batch culture	UdhA overexpression	Final PHB—3.52 g/L Specific PHB content—0.49 g PHB/g cell	Final PHB—6.42 g/L Specific PHB content—0.66 g PHB/g cell	Sánchez et al. (2006)
	Glucose	Thymidine	Glucose	Aerobic pH-stat fed-batch culture	UdhA overexpression	Final thymidine—1.02 g/L Productivity—15.8 mg/L h Yield—6.1 mg thymidine/g glucose	Final thymidine—1.92 g/L Productivity—30.1 mg/L h Yield—13 mg thymidine/g glucose	Lee et al. (2010)
	Glucose	Isobutanol	Glucose	Anaerobic batch culture	PntAB overexpression	Final isobutanol—1.3 g/L Yield—0.21 g isobutanol/g glucose	Final isobutanol—8.5 g/L Yield—0.40 g isobutanol/g glucose	Bastian et al. (2011)
	Glucose	Isobutanol	Glucose	Aerobic batch culture	Chromosomal modulation of PntAB expression	Final isobutanol—8.4 g/L Yield—0.21 g isobutanol/g glucose	Final isobutanol—10.2 g/L Yield—0.24 g isobutanol/g glucose	Shi et al. (2013)
	Glucose	Isobutanol	Glucose	Anaerobic batch culture	Chromosomal modulation of PntAB expression	Final isobutanol—2.6 g/L Yield—0.27 g isobutanol/g glucose	Final isobutanol—3.1 g/L Yield—0.29 g isobutanol/g glucose	
	Glucose	3-HP	Glucose	Aerobic batch culture	PntAB overexpression	Final 3-HP—1.6 mM Yield—0.02 mol 3-HP/mol glucose	Final 3-HP—2.14 mM Yield—0.03 mol 3-HP/mol glucose	Rathnasingh et al. (2012)
Generation of NADPH during glycolysis	Glucose	Lycopene	Glucose	Aerobic batch culture	Replacement of native GapA with GapC from *C. acetobutylicum*	Final lycopene—0.4 g/L Productivity—16.7 mg/L h	Final thymidine—1.0 g/L Productivity—41.7 mg/L h	Mártinez et al. (2008)
	Glucose	PHB	Glucose	Aerobic batch culture	YfjB overexpression	Final PHB—7 g/L Specific PHB content—0.34 g PHB/g cell Yield—0.08 g PHB/g glucose	Final PHB—14 g/L Specific PHB content—0.50 g PHB/g cell Yield—0.15 g PHB/g glucose	Li et al. (2009)
	Glycerol	Thymidine	Glycerol	Aerobic batch culture	YfjB overexpression	Final thymidine—0.74 g/L Productivity—21.1 mg/L h Yield—12.3 mg thymidine/g glycerol	Final thymidine—0.94 g/L Productivity—26.9 mg/L h Yield—15.7 mg thymidine/g glycerol	Lee et al. (2009)
	Glucose	Thymidine	Glucose	Aerobic pH-stat fed-batch culture	YfjB overexpression	Final thymidine—1.02 g/L Productivity—15.8 mg/L h Yield—6.1 mg thymidine/g glucose	Final thymidine—1.41 g/L Productivity—22.5 mg/L h Yield—9.1 mg thymidine/g glucose	Lee et al. (2010)

Table 2 (continued)

Approaches	Substrates	Products	Carbon sources for NADPH	Culture conditions	Genetic manipulations	Key features		References
						Control	Experimental	
	Glucose	Isobutanol	Glucose	Aerobic batch culture	Chromosomal modulation of YfjB expression	Final isobutanol—8.4 g/L Yield—0.21 g isobutanol/g glucose	Final isobutanol—8.9 g/L Yield—0.21 g isobutanol/g glucose	Shi et al. (2013)
	Glucose	Isobutanol	Glucose	Anaerobic batch culture	Chromosomal modulation of YfjB expression	Final isobutanol—2.6 g/L Yield—0.27 g isobutanol/g glucose	Final isobutanol—2.7 g/L Yield—0.29 g isobutanol/g glucose	
	Glucose	Isobutanol	Glucose	Aerobic batch culture	Combinatorial modulation of PntAB and YfjB expression (NADH kinase-like effect)	Final isobutanol—8.4 g/L Yield—0.21 g isobutanol/g glucose	Final isobutanol—10.8 g/L Yield—0.26 g isobutanol/g glucose	
	Glucose	Isobutanol	Glucose	Anaerobic batch culture	Combinatorial modulation of PntAB and YfjB expression (NADH kinase-like effect)	Final isobutanol—2.6 g/L Yield—0.27 g isobutanol/g glucose	Final isobutanol—4.7 g/L Yield—0.38 g isobutanol/g glucose	
	Glucose	GDP-L-fucose	Glucose	Aerobic pH-stat fed-batch culture	Overexpression of Pos5p from *S. cerevisiae*	Final GDP-L-fucose—193.6 mg/L Productivity—10.2 mg/L h Yield—1.0 mg GDP-L-fucose/g glucose	Final GDP-L-fucose—291.5 mg/L Productivity—13.9 mg/L h Yield—1.4 mg GDP-L-fucose/g glucose	Lee et al. (2013)

phenylethanol and xylitol (Johannes et al. 2006). It also worked well for oxidation of indigo compounds and sulfoxidation of prochiral sulfides (Rioz-Martínez et al. 2011). The engineered PTDH has not been used in whole-cell-based bioprocess yet; however, it is expected to conduct NADPH regeneration efficiently because PTDH is not involved in the central metabolic pathway (Fig. 1) (Johannes et al. 2006; Rioz-Martínez et al. 2011).

Concluding remarks

Whole-cell-based biotransformation processes for production of value-added materials have developed drastically through engineering of cellular metabolic pathways and process optimization. Since the overall productivity of the whole-cell biotransformation relies on the efficiency of the enzymes participating in the cofactors requiring reactions, cofactors such as NADPH should be supplied sufficiently in order to sustain cellular metabolism and biotransformation reactions. Hence, a number of metabolic engineering studies have focused on increasing intracellular NADPH availability in biotransformation processes.

This review summarized several metabolic pathways used for intracellular NADPH regeneration in *E. coli* and discussed the progress of those studies. Modulation of the PPP (overexpression of G6PDH or introduction of GDH) and overexpression of transhydrogenases (UdhA and PntAB) have been frequently used as primary solutions for NADPH regeneration because these approaches have shown beneficial effects on NADPH-dependent processes. Meanwhile, the fact that NADH can be easily generated through several pathways compared with NADPH has been a driving force for modulating the cellular metabolism to produce NADPH during glycolysis. As an alternative approach, introduction of NADH kinase from *S. cerevisiae* and replacement of native GAPDH with NADPH-producing GAPDH from *C. acetobytilicum* were attempted to regenerate NADPH during glycolysis and showed significant impacts on NADPH-dependent bioprocesses in terms of final product concentration and productivity. Because engineering of the pathways related with redox balance of NADPH may cause unknown metabolic perturbations, more research should be performed to minimize side effects by modulation of the intracellular NADPH generating pathways and to maximize the productivity of target compounds through optimization of cellular conditions.

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