Dynamic model of *E. coli* nitrogen assimilation system

Biochemical network of *E. coli* nitrogen assimilation system

E. coli absolutely needs ammonia for synthesizing glutamine and glutamate, which are the primary products of ammonia assimilation, from which almost all nitrogen-containing compounds, including amino acids and nucleotides, derive. Glutamine and glutamate are synthesized through glutamine synthetase (GS), glutamate synthase (GOGAT), and glutamate dehydrogenase (GDH) by adding ammonia to 2-ketoglutarate that is an intermediate of the TCA cycle. Among these enzymes, GS plays a critical role in connecting nitrogen metabolism to carbon metabolism (Ninfa et al. 2000). In order to maintain the balanced metabolism between nitrogen and carbon, multiple negative and positive feedback loops of the nitrogen assimilation system control the activity and synthesis of *glnA* (GS) and the transcription of nitrogen-regulated (*Ntr*) genes, *glnG* (NRI), *glnL* (NRII), *glnK* (GlnK), and *nac*, whose products facilitate the adaptation to ammonia deficiency, monitoring the concentrations of nitrogenous and carboneous components.

The activity of GS is controlled in at least three ways. First, the enzyme is subjected to concerted feedback inhibition by various nitrogenous compounds such as amino acids. Second, the GS activity is regulated by reversible covalent adenylylation. Finally, the level of GS is regulated at the rate for transcription of its structural gene, *glnA*. These three mechanisms achieve the rapid, precise, efficient, and broad-range regulation of GS activity in response to signals of nitrogenous and carboneous sources. In this paper, we focus on the system that consists of the reversible adenylylation and transcription of *glnA*.

Such a regulation system is decomposed into three monocyclic systems: (i) PII-UTase/UR monocycle, (ii) GS-PI (ATase) monocycle, (iii) NRI-NRII monocycle (positive feedback loop). The UTase/UR and PII bicyclic system is composed of (i) and (ii) regulates the activity of glutamine synthetase. UTase/UR and NRI-NRII bicyclic system composed of (i) and (iii) is responsible for controlling the synthesis of GS and *Ntr* genes. These two bicyclic systems constitute major feedback loops for controlling the activity and synthesis of GS, respectively.

The adenylylation of GS is controlled by glutamine and 2-ketoglutarate in the bicyclic and monocyclic cascades that consist of PI, PII, and UTase/UR proteins. In the bicyclic cascade, PI not only catalyzes the ATP-dependent addition of AMP to a subunit of GS, with the release of PPi, but also catalyzes the phosphate-dependent removal of AMP from each subunit of GS. These two inverse reactions depend on whether PII is uridylylated. The uridylylation of PII is controlled by the bifunctional UTase/UR, which catalyzes the covalent uridylylation or deuridylylation of PII, monitoring glutamine and 2-ketoglutarate. When 2-ketoglutarate binds to UTase/UR, UTase catalyzes the UTP-dependent addition of UMP to PII. PII-UMP stimulates the deadenylylating activity of PI, resulting in deadenlylating GS. When glutamine binds to UTase/UR, UR catalyzes deuridylylation of PII-UMP and unmodified PI makes PII adenylylate GS. GS activity is controlled by the symmetric adenylylation/deadenylylation feedback loop. In the monocyclic cascade, 2-ketoglutarate controls PII directly. PII that binds to 2-ketoglutarate does not activate the adenylylation activity of ATase, showing that 2-ketoglutarate controls the activity of PII without UTase/UR.

The synthesis of GS is controlled by the other bicyclic cascade loop composed of key components, UTase/UR,

PII, NRI, NRII and σ^{54}. UTase/UR and PII sense the concentrations of glutamine and 2-ketogluatarate, resulting in regulation of the transcription of the *glnALG* operon encoding *glnA* (GS), *glnL* (NRII), and *glnG* (NRI) and other Ntr proteins, including proteins responsible for transporting and degrading nitrogenous compounds.

In the branch point of the dual bicyclic cascades, PII, which shows the activity for binding unphosphorylated NRII, is a signal that transduces the information regarding glutamine and 2-ketoglutarate to the cascade of the GS synthesis (Ninfa and Atkinson 2000). GlnK is a PII homolog in function, but its transcription regulation is governed by NRI-P, whereas PII is synthesized constitutively (Blauwkamp and Ninfa 2002). NRII shows the reciprocal regulation of the kinase and phosphatase activities. Without PII nor GlnK, the autophosphorylated NRII can transfer its phosphate to NRI. NRI is phosphorylated not only by phosphorylated NRII but also directly by acetylphosphate. Dephosphorylated NRII controls the dephosphorylation of NRI-P. NRI is a transcriptional suppressor that controls in a σ^{70} dependent promoter. NRI-phosphate forms multimers, strongly activating the expression of the *glnALG* operon and other *Ntr* genes (*glnK*, *nac*) in a σ^{54} dependent manner, resulting in a higher level of GS, NRII and NRI. This forms not only negative feedback loops (NRI, GS, UTase/UR, PII, NRII), but also positive feedback loops (NRI, NRII), thereby increasing the activity of NRI-P.

References

Blauwkamp, T.A. and A.J. Ninfa. 2002. Physiological role of the GlnK signal transduction protein of Escherichia coli: survival of nitrogen starvation. *Mol Microbiol* **46:** 203-214.

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Supplementary Table 1 Regulator-reaction equations of the *E. coli* nitrogen assimilation system. The arrows indicate irreversible reaction (>), reversible reaction (<->), catalysis (-()), activation (->>), inhibition (-||), and degradation (> &). The symbols of ":" and "-" indicate binding complexes and modification, respectively. For example, the molecules of PI:PII, NRI-P:Enhancer, and NRI-P:NRII are binding complexes, whereas NRI-P is phosphorylated NRI. The naming rules are described in Supplemental Method 1.

Metabolic layer
GS -() glutamate + ammonia > glutamine GOGAT -() glutamine + 2-ketoglutarate > 2glutamate GDH -() 2-ketoglutarate + ammonia > glutamate Dummy > 2-ketoglutarate glutamine > & glutamate > & 2-ketoglutarate > &
Protein layer
UTase/UR + 2-ketoglutarate <-> UTase/UR:2-ketoglutarate UTase/UR + glutamine <-> UTase/UR:glutamine UTase/UR:2-ketoglutarate -() PII + UMP > PII-UMP UTase/UR:2-ketoglutarate -() GlnK + UMP > GlnK-UMP UTase/UR:glutamine -() PII-UMP > PII + UMP UTase/UR:glutamine -() GlnK-UMP > GlnK + UMP PII + PI <-> PII:PI GlnK + PI <-> GlnK:PI PII-UMP + PI <-> PII-UMP:PI GlnK-UMP + PI <-> GlnK-UMP:PI PII:PI -() GS + AMP > GS-AMP GlnK:PI -() GS + AMP > GS-AMP PII-UMP:PI -() GS-AMP > GS + AMP GlnK-UMP:PI -() GS-AMP > GS + AMP NRII + PII <-> NRII:PII NRII + GlnK <-> NRII:GlnK

NRII:PII -() NRI-P > NRI + P
NRII:GlnK -() NRI-P > NRI + P
NRII + ATP <-> NRII:ATP
NRII:ATP > NRII-P
NRII-P > NRII + P
PII -() NRII-P + ADP > NRII + ATP
GlnK -() NRII-P + ADP > NRII + ATP
NRI + NRII-P <-> NRI:NRII-P
NRI:NRII-P > NRI-P + NRII
NRI + acetylphosphate <-> NRI:acetylphosphate
NRI:acetylphosphate > NRI-P + acetylphospate
NRI + Promoter(glnAp1) <-> NRI:Promoter(glnAp1)
NRI + Promoter(glnLp) <-> NRI:Promoter(glnLp)
4NRI-P + Enhancer(glnAp2) <-> $(NRI\text{-}P)_4$:Enhancer(glnAp2)
NRI-P + Enhancer(GlnK) <-> NRI-P:Enhancer(GlnK)
NRI-P + Enhancer(nac) <-> NRI-P:Enhancer(nac)

Gene layer

NRI:Promoter(glnAp1) -|| DNA(GS) -> mRNA(GS)
NRI:Promoter(glnLp) -|| DNA(NRI) -> mRNA(NRI)
NRI:Promoter(glnLp) -|| DNA(NRII) -> mRNA(NRII)
$(NRI\text{-}P)_4$:Enhancer(glnAp2) ->> DNA(GS) -> mRNA(GS)
$(NRI\text{-}P)_4$:Enhancer(glnAp2) ->> DNA(NRI) ->mRNA(NRI)
$(NRI\text{-}P)_4$:Enhancer(glnAp2) ->> DNA(NRII) -> mRNA(NRII)
NRI-P:Enhancer(GlnK) ->> DNA(GlnK) -> mRNA(GlnK)
NRI-P:Enhancer (Nac) ->> DNA(nac) -> mRNA(nac)
mRNA(GS) -> GS
mRNA(NRI) -> NRI
mRNA(NRII) -> NRII
mRNA(GlnK) -> GlnK
mRNA(nac) -> nac

Supplementary Table 2 Full mathematical equations of the *E. coli* nitrogen assimilation system. The suffixes of "f" and "o" indicate free and total concentrations, respectively. The square brackets [ ] mean the molar concentration.

Binding Phase
[*UTase/UR:2-ketoglutarate*] = *K[1]*[*UTase/UR*][*2-ketoglutarate*]
[*UTase/UR:glutamine*] = *K[2]*[*UTase/UR*][*glutamine*]
[*PII:UMP:UTase/UR:2-ketoglutarate*] = *K[3]*[*UTase/UR:2-ketoglutarate*][*PII*][UMP]
[*GlnK:UMP:2-ketoglutarate:UTase/UR*] = *K[4]*[*GlnK*][*UTase/UR:2-ketoglutarate*][*UMP*]
[*UTase/UR:glutamine:PII-UMP*] = *K[5]*[*UTase/UR:glutamine*][*PII-UMP*]
[*GlnK-UMP:glutamine:UTase/UR*] = *K[6]*[*GlnK-UMP*][*UTase/UR:glutamine*]
[*PI:PII*] = *K [7]*[*PI*][*PII*]
[*GlnK:PI*] = *K[8]*[*GlnK*][*PI*]
[*PI:PII-UMP*] = *K[9]*[*PI*][*PII-UMP*]
[*GlnK-UMP:PI*] = *K[10]*[*GlnK-UMP*][*PI*]
[*GS:AMP:PI:PII*] = *K [11]*[*PI:PII*][*GS*][*AMP*]
[*GS:AMP:PI:GlnK*] = *K[12]*[*GS*][*GlnK:PI*][*AMP*]
[*GS-AMP:PI:PII-UMP*] = *K [13]*[*PI:PII-UMP*][*GS-AMP*]
[*GS-AMP:PI:GlnK-UMP*] = *K[14]*[*GlnK-UMP:PI*][*GS-AMP*]
[*NRII:PII*] = *K [15]*[*NRII*][*PII*]
[*NRII:GlnK*] = *K[16]*[*GlnK*][*NRII*]
[*NRII:ATP*] = *K[17]*[*NRII*][*ATP*]
[*NRI:NRII-P*] = *K[18]*[*NRI*][*NRII-P*]
[NRI:acetylphosphate] = *K [19]*[*NRI*][*acetylphosphate*]
[*NRI-P:NRII:PII*] = *K [20]*[*NRII:PII*][*NRI-P*]
[*NRI-P:NRII:GlnK*] = *K[21]*[*NRI-P*][*NRII:GlnK*]
[*NRII-P:PII:ADP*] = *K [22]*[*PII*][*NRII-P*][*ADP*]
[*NRII-P:ADP:GlnK*] = *K[23]*[*NRII-P*][*GlnK*][*ADP*]
[*NRI:Promoter(glnAp1)*] = *K[24]*[*NRI*][*Promoter(glnAp1)*]
[*NRI:Promoter(glnLp)*] = *K[25]*[*NRI*][*Promoter(glnLp)*]
[*NRI-P(4):Enhancer(glnAp2)*] = *K[26]* ^{4} [*NRI-P*] ^{4} [*Enhancer(glnAp2)*]
[*NRI-P:Enhancer(GlnK)*] = *K[27]*[*NRI-P*][*Enhancer(GlnK)*]
[*NRI-P:Enhancer(nac)*] = *K[28]*[*Enhancer(nac)*][*NRI-P*]

$[UTase/UR]_o = [UTase/UR] + [UTase/UR:glutamine] + [UTase/UR:2\text{-}ketoglutarate]$
$+ [PII:UMP:UTase/UR:2\text{-}ketoglutarate] + [UTase/UR:glutamine:PII\text{-}UMP]$
$+ [UTase/UR:glutamine:GlnK\text{-}UMP] + [GlnK:UMP:UTase/UR:2\text{-}ketoglutarate]$

$[PII]_o = [PII] + [PII:UMP:UTase/UR:2\text{-}ketoglutarate] + [PI:PII] + [GS:AMP:PI:PII] + [NRII:PII]$
$+ [NRII\text{-}P:PII:ADP] + [NRI\text{-}P:NRII:PII]$

$[PII\text{-}UMP]_o = [PII\text{-}UMP] + [UTase/UR:glutamine:PII\text{-}UMP] + [PI:PII\text{-}UMP] + [GS\text{-}AMP:PI:PII\text{-}UMP]$

$[GlnK]_o = [GlnK] + [GlnK:PI] + [NRII:GlnK] + [GS:AMP:PI:GlnK]$
$+ [GlnK:UMP:2\text{-}ketoglutarate:UTase/UR] + [NRII\text{-}P:ADP:GlnK] + [NRI\text{-}P:NRII:GlnK]$

$[GlnK\text{-}UMP]_o = [GlnK\text{-}UMP] + [GlnK\text{-}UMP:PI] + [GS\text{-}AMP:PI:GlnK\text{-}UMP]$
$+ [GlnK\text{-}UMP:glutamine:UTase/UR]$

$[PI]_o = [PI] + [PI:PII] + [PI:PII\text{-}UMP] + [GS:AMP:PI:PII] + [GS\text{-}AMP:PI:PII\text{-}UMP] + [GlnK:PI]$
$+ [GS:AMP:GlnK:PI] + [GlnK\text{-}UMP:PI] + [GS\text{-}AMP:GlnK\text{-}UMP:PI]$

$[GS]_o = [GS] + [GS:AMP:PI:PII] + [GS:AMP:PI:GlnK]$

$[GS\text{-}AMP]_o = [GS\text{-}AMP] + [GS\text{-}AMP:PI:PII\text{-}UMP] + [GS\text{-}AMP:PI:GlnK\text{-}UMP]$

$[NRII]_o = [NRII] + [NRII:PII] + [NRII:ATP] + [NRI\text{-}P:NRII:PII] + [NRII:GlnK] + [NRI\text{-}P:NRII:GlnK]$

$[NRII\text{-}P]_o = [NRII\text{-}P] + [NRII\text{-}P:PII:ADP] + [NRI:NRII\text{-}P] + [NRII\text{-}P:GlnK:ADP]$

$[NRI]_o = [NRI] + [NRI:NRII\text{-}P] + [NRI:acetylphosphate] + [NRI:Promoter(glnAp1)]$
$+ [NRI:Promoter(glnLp)]$

$[NRI\text{-}P]_o = [NRI\text{-}P] + [NRI\text{-}P:NRII:PII] + 4[NRI\text{-}P(4):Enhancer(glnAp2)]$
$+ [NRI\text{-}P:NRII:GlnK] + [NRI\text{-}P:Enhancer(nac)]$

$[Promoter(glnAp1)]_o = [Promoter(glnAp1)] + [NRI:Promoter(glnAp1)]$

$[Promoter(glnLp)]_o = [Promoter(glnLp)] + [NRI:Promoter(glnLp)]$

$[Enhancer(glnAp2)]_o = [Enhancer(glnAp2)] + [NRI\text{-}P(4):Enhancer(glnAp2)]$

$[Enhancer(GlnK)]_o = [Enhancer(GlnK)] + [NRI\text{-}P:Enhancer(GlnK)]$

$[Enhancer(nac)]_o = [Enhancer(nac)] + [NRI\text{-}P:Enhancer(nac)]$

Reaction Phase

$$\frac{d[mRNA(GS)]}{dt} = +km[2]([NRI\text{-}P(4):Enhancer(glnAp2)]/[Enhancer(glnAp2)]_o)\cdot[DNA(GS)]$$
$$+(1-([NRI:Promoter(glnAp1)]/[Promoter(glnAp1)]_o))\cdot[DNA(GS)]$$
$$-kmd[2][mRNA(GS)]$$

$$\frac{d[mRNA(GlnK)]}{dt} = +km[4]([NRI\text{-}P:Enhancer(GlnK)]/[Enhancer(GlnK)]_o)[DNA(GlnK)]$$
$$-kmd[4][mRNA(GlnK)]$$

$$\frac{d[mRNA(NRII)]}{dt} = +km[1]([NRI\text{-}P(4):Enhancer(glnAp2)]/[Enhancer(glnAp2)]_o)\cdot[DNA(NRII)]$$
$$+(1-([NRI:Promoter(glnLp)]/[Promoter(glnLp)]_o))\cdot[DNA(NRII)]$$
$$-kmd[1][mRNA(NRII)]$$

$$\frac{d[mRNA(NRI)]}{dt} = +km[3]([NRI\text{-}P(4):Enhancer(glnAp2)]/[Enhancer(glnAp2)]_o)\cdot[DNA(NRI)]$$
$$+(1-([NRI:Enhancer(glnLp)]/[Enhancer(glnLp)]_o))\cdot[DNA(NRI)]$$
$$-kmd[3][mRNA(NRI)]$$

$$\frac{d[mRNA(nac)]}{dt} = +km[5]([NRI\text{-}P:Enhancer(nac)]/[Enhancer(nac)]_o)[DNA(nac)]$$
$$-kmd[5][mRNA(nac)]$$

$$\frac{d[GS]_o}{dt} = -kx[10][GS:AMP:PI:PII]-kpd[15][GS:AMP:PI:PII]+kx[12][GS\text{-}AMP:PI:PII\text{-}UMP]$$
$$+kp[2][mRNA(GS)]-kpd[7][GS]+kx[11][GS\text{-}AMP:PI:GlnK\text{-}UMP]$$
$$-kx[9][GS:AMP:PI:GlnK]-kpd[17][GS:AMP:PI:GlnK]$$

$$\frac{d[GS\text{-}AMP]_o}{dt} = -kx[12][GS\text{-}AMP:PI:PII\text{-}UMP]-kpd[19][GS\text{-}AMP:PI:PII\text{-}UMP]$$
$$+kx[10][GS:AMP:PI:PII]+kx[9][GS:AMP:PI:GlnK]$$
$$-kx[11][GS\text{-}AMP:PI:GlnK\text{-}UMP]-kpd[8][GS\text{-}AMP]$$
$$-kpd[21][GS\text{-}AMP:PI:GlnK\text{-}UMP]$$

$$\frac{d[PII]_o}{dt} = -kx[5][PII:UMP:UTase/UR:2\text{-}ketoglutarate]+kx[7][UTase/UR:glutamine:PII\text{-}UMP]$$

$$\frac{d[PII\text{-}UMP]_o}{dt} = -kx[7][UTase/UR:glutamine:PII\text{-}UMP]+kx[5][PII:UMP:UTase/UR:2\text{-}ketoglutarate]$$

$$\frac{d[GlnK]_o}{dt} = +kp[4][mRNA(GlnK)]+kx[8][GlnK\text{-}UMP:glutamine:UTase/UR]-kpd[5][GlnK]$$
$$-kpd[16][GlnK:PI]-kpd[24][NRII:GlnK]-kx[6][GlnK:UMP:2\text{-}ketoglutarate:UTase/UR]$$
$$-kpd[17][GS:AMP:GlnK:PI]-kpd[26][NRI\text{-}P:NRII:GlnK]$$
$$-kpd[30][GlnK:UMP:2\text{-}ketoglutarate:UTase/UR]-kpd[34][NRII\text{-}P:ADP:GlnK]$$

$$\frac{d[GlnK\text{-}UMP]}{dt} = +kx[6][GlnK:UMP:2-ketoglutarate:UTase/UR]$$
$$-kpd[21][GS\text{-}AMP:GlnK\text{-}UMP:PI]$$
$$-kpd[20][GlnK\text{-}UMP:PI]-kx[8][GlnK\text{-}UMP:glutamine:UTase/UR]$$
$$-kpd[6][GlnK\text{-}UMP]-kpd[22][GlnK\text{-}UMP:glutamine:UTase/UR]$$

$$\frac{d[NRII]_o}{dt} = -kpd[23][NRII:PII]-kx[15][NRII:ATP]-kpd[32][NRII:ATP]+kx[16][NRII\text{-}P:PII:ADP]$$
$$+kx[13][NRI:NRII\text{-}P]+kp[1][mRNA(NRII)]-kpd[3][NRII]-kpd[25][NRI\text{-}P:NRII:PII]$$
$$+kx[17][NRII\text{-}P:ADP:GlnK]-kpd[24][NRII:GlnK]+kpd[26][NRI\text{-}P:NRII:GlnK]$$

$$\frac{d[NRII\text{-}P]_o}{dt} = -kx[16][NRII\text{-}P:PII:ADP]-kpd[33][NRII\text{-}P:PII:ADP]-kx[13][NRI:NRII\text{-}P]$$
$$-kpd[28][NRI:NRII\text{-}P]+kx[15][NRII:ATP]-kpd[4][NRII\text{-}P]$$
$$-kx[17][NRII\text{-}P:ADP:GlnK]-kpd[34][NRII\text{-}P:ADP:GlnK]$$

$$\frac{d[NRI]_o}{dt} = -kx[13][NRI : NRII - P] - kpd[28][NRI : NRII - P] - kx[14][NRI : acetlyphosphate]$$
$$- kpd[31][NRI : acetlyphosphate] - kpd[10][NRI : Promoter(glnAp1)]$$
$$- kpd[12][NRI : Promoter(glnLp)] + kx[1][NRI - P : NRII : PII] + kp[3][mRNA(NRI)]$$
$$- kpd[1][NRI] + kx[2][NRI - P : NRII : GlnK]$$

$$\frac{d[NRI - P]_o}{dt} = -kx[1][NRI - P : NRII : PII] - kpd[25][NRI - P : NRII : PII] + kx[13][NRI : NRII - P]$$
$$+ kx[14][NRI : acetlyphosphate] - kdp[11] \cdot 4[NRI - P(4) : Enhancer(glnAp2)] - kpd[2][NRI - P]$$
$$- kpd[27][NRI - P : Enhancer(GlnK)] - kx[2][NRI - P : NRII : GlnK]$$
$$- kpd[26][NRI - P : NRII : GlnK] - kpd[29][NRI - P : Enhancer(nac)]$$

$$\frac{d[nac]}{dt} = +kp[5][mRNA(nac)] - kpd[9][nac]$$

(Metabolic reactions)

$$\frac{d[glutamine]}{dt} = +Q[5][GS]\frac{[ammonia]}{Km[4]+[ammonia]}\frac{[glutamate]}{Km[5]+[glutamate]}$$
$$-0.5Q[6][GOGAT]\frac{[glutamine]}{Km[6]+[glutamine]}\frac{[2 - ketoglutarate]}{Km[7]+[2 - ketoglutarate]}$$
$$-Q[1]\frac{[glutamine]}{Km[10]+[glutamine]}$$

$$\frac{d[glutamate]}{dt} = +Q[6][GOGAT]\frac{[glutamine]}{Km[6]+[glutamine]}\frac{[2 - ketoglutarate]}{Km[7]+[2 - ketoglutarate]}$$
$$+Q[7][GDH]\frac{[ammonia]}{Km[8]+[ammonia]}\frac{[2 - ketoglutarate]}{Km[9]+[2 - ketoglutarate]}$$
$$-Q[2]\frac{[glutamate]}{Km[2]+[glutamate]}$$
$$-Q[5][GS]\frac{[ammonia]}{Km[4]+[ammonia]}\frac{glutamate]}{Km[5]+[glutamate]}$$

$$\frac{d[2 - ketoglutarate]}{dt} = +Q[3][Dummy]\frac{[Dummy]}{Km[11]+[Dummy]}$$
$$-Q[4]\frac{[2 - ketoglutarate]}{Km[3]+[2 - ketoglutarate]}$$
$$-0.5Q[6][GOGAT]\frac{[glutamine]}{Km[6]+[glutamine]}\frac{[2 - ketoglutarate]}{Km[7]+[2 - ketoglutarate]}$$
$$-Q[7][GDH]\frac{[ammonia]}{Km[8]+[ammonia]}\frac{[2 - ketoglutarate]}{Km[9]+[2 - ketoglutarate]}$$

Supplemental Table 3. Parameters for a full mathematical model of the *E. coli* nitrogen assimilation system.

(a) Components used in the model. The symbols of ":" and "-" indicate binding complexes and modification, respectively. For example, the molecules of PI:PII, NRI-P:Enhancer, and NRI-P:NRII are binding complexes, whereas NRI-P is phosphorylated NRI.

Component	**Definition**	**Concentration**
UTase/UR	UTase/UR	8.5247×10^{-4} [nM]
PII	PII	4.9724×10^{-1} [nM]
PII-UMP	uridylylated PII	1.9496×10^{-2} [nM]
GlnK	GlnK	3.9254×10^{-3} [nM]
GlnK-UMP	uridylylated GlnK	1.5233×10^{-4} [nM]
GS	glutamine synthetase	2.8024 [nM]
GS-AMP	adenylylated GS	3.6782×10^{-1} [nM]
GOGAT	glutamate synthase	1.00×10^{2} [nM]
GDH	glutamine dehydrogenase	1.00×10^{2} [nM]
PI	PI, adenylyltransferase	4.8327 [nM]
NRI	NRI	1.0811 [nM]
NRI-P	phosphorylated NRI	1.3201×10 [nM]
NRII	NRII	9.0845×10^{-4} [nM]
NRII-P	phosphorylated NRII	6.5435×10^{-2} [nM]
nac	one of the Ntr proteins	3.0333 [nM]
Promoter(glnAp1)	enhancer for GS	2.1760×10^{-2} [nM]
Promoter(glnLp)	promoter for NRI and NRII	3.3448×10^{-1} [nM]
Enhancer(glnAp2)	enhancer for GS, NRI, and NRII	9.9944×10^{-1} [nM]
Enhancer(GlnK)	enhancer for GlnK	9.9860×10^{-1} [nM]
Enhancer(nac)	enhancer for nac	9.7877×10^{-1} [nM]
AMP		1.00×10^{7} [nM]
ADP		1.00×10^{7} [nM]
ATP		1.00×10^{7} [nM]
UMP		1.00×10^{7} [nM]
mRNA(GS)	mRNA of GS	5.5801×10^{-3} [nM]
mRNA(NRI)	mRNA of NRI	8.3758×10^{-2} [nM]
mRNA(NRII)	mRNA of NRII	8.3758×10^{-2} [nM]
mRNA(GlnK)	mRNA of GlnK	1.0495×10^{-3} [nM]
mRNA(nac)	mRNA of nac	5.3082×10^{-3} [nM]
DNA(GS)	gene encoding GS (*glnA*)	1.00 [nM]
DNA(NRI)	gene encoding NRI (*glnG*)	1.00 [nM]

DNA(NRII)	gene encoding NRII (*glnL*)	1.00 [nM]
DNA(GlnK)	gene encoding GlnK (*glnK*)	1.00 [nM]
DNA(nac)	gene encoding of nac	1.00 [nM]
ammonia	NH_3	1.0×10^{5} [nM]
2-ketoglutarate		2.2917×10^{5} [nM]
glutamine		2.3376×10^{5} [nM]
glutamate		8.2392×10^{4} [nM]
acetylphosphate	acetylphosphate	1.00×10^{3}[nM]
UTase/UR:glutamine	glutamine-bound UTase/UR	3.6916×10^{2} [nM]
UTase/UR:2-ketoglutarate	2-ketoglutarate -bound UTase/UR	2.8668×10^{2} [nM]
PII:UMP:UTase/UR:2-ketoglutarate	PII:UMP:UTase/UR: 2-ketoglutarate	1.9526×10 [nM]
UTase/UR:glutamine:PII-UMP	UTase/UR:glutamine:PII-UMP	1.9526×10 [nM]
PI:PII	PI:PII	2.2759×10 [nM]
PI:PII-UMP	PI:PII-UMP	1.4084×10^{-2} [nM]
NRII:PII	NRII:PII	3.1095×10^{-2} [nM]
NRII:ATP	NRII:ATP	1.5981×10 [nM]
NRII-P:PII:ADP	NRII-P:PII:ADP	3.3004×10^{-4} [nM]
NRI:NRII-P	NRI:NRII-P	1.5901×10 [nM]
NRI:acetylphosphate	acetylphosphate-bound NRI	1.2750×10^{-1} [nM]
NRI-P:NRII:PII	NRI-P:NRII:PII	1.5758×10 [nM]
NRI:Promoter(glnAp1)	NRI-bound promoter(glnAp1)	9.7824×10^{-1} [nM]
NRI:Promoter(glnLp)	NRI-bound promoter(glnLp)	6.6552×10^{-1} [nM]
NRI-P(4):Enhancer(glnAp2)	NRI-P(4)-bound enhancer(glnAp2)	5.6130×10^{-4} [nM]
GS:AMP:PI:PII	GS:AMP:PI:PII	1.0057×10^{-2} [nM]
GS-AMP:PI:PII-UMP	GS-AMP:PI:PII-UMP	8.1923×10^{-3} [nM]
NRII:GlnK	NRII:GlnK	2.4548×10^{-4} [nM]
GS-AMP:PI:GlnK-UMP	GS-AMP:PI:GlnK-UMP	6.4009×10^{-5} [nM]
GS:AMP:PI:GlnK	GS:AMP:PI:GlnK	7.9398×10^{-5} [nM]
NRII-P:ADP:GlnK	NRII-P:ADP:GlnK	2.6055×10^{-6} [nM]
NRI-P:NRII:GlnK	NRI-P:NRII:GlnK	1.2440×10^{-1} [nM]
GlnK:UMP:2-ketoglutarate:UTase/UR	GlnK:UMP:2-ketoglutarate:UTase/UR	1.5415×10^{-1} [nM]
GlnK-UMP:glutamine:UTase/UR	GlnK-UMP:glutamine:UTase/UR	1.5256×10^{-1} [nM]
GlnK-UMP:PI	GlnK-UMP:PI	1.6401×10^{-1} [nM]
GlnK:PI	GlnK:PI	1.1119×10^{-4} [nM]
NRI-P:Enhancer(GlnK)	NRI-P-bound enhancer(GlnK)	1.3993×10^{-3} [nM]
NRI-P:Enhancer(nac)	NRI-P-bound enhancer(nac)	2.1233×10^{-2} [nM]
Dummy	assumed precursor for 2-ketoglutarate	1.00×10^{5} [nM]

(b) Biochemical parameters used in the model

S: the parameters for the GS synthesis feedback module, which are estimated by GAs, **A**: the parameters for the GS activity feedback module, which are estimated by GAs, **B**: the values provided or assumed based on biological data (literatures), **E**: the values assumed in the model.

Parameter	Definition	Value	Class
K[1]	association constant between 2-ketoglutarate and UTase/UR	1.467×10^{9} [M^{-1}]	**A/S**
K[2]	association constant between glutamine and UTase/UR	1.852×10^{9} [M^{-1}]	**A/S**
K[3]	association constant between PII, UMP and 2-ketoglutarate:UTase/UR	1.369×10^{10} [M^{-2}]	**A/S**
K[4]	association constant between GlnK, UMP and UTase/UR:2-ketoglutarate	1.369×10^{10} [M^{-2}]	**A/S**
K[5]	association constant between PII-UMP and UTase/UR:glutamine	2.713×10^{9} [M^{-1}]	**A/S**
K[6]	association constant between GlnK-UMP and UTase/UR:glutamine	2.713×10^{9} [M^{-1}]	**A/S**
K[7]	association constant between PII and PI	5.861×10^{6} [M^{-1}]	**A**
K[8]	association constant between GlnK and PI	5.861×10^{6} [M^{-1}]	**A**
K[9]	association constant between PII-UMP and PI	2.227×10^{11} [M^{-1}]	**A**
K[10]	association constant between GlnK-UMP and PI	2.227×10^{11} [M^{-1}]	**A**
K[11]	association constant between GS, AMP and PI:PII	2.548×10^{10} [M^{-2}]	**A**
K[12]	association constant between GS, AMP and GlnK:PI	2.548×10^{10} [M^{-2}]	**A**
K[13]	association constant between GS-AMP and PI:PII-UMP	1.061×10^{6} [M^{-1}]	**A**
K[14]	association constant between GS-AMP and GlnK-UMP :PI	1.061×10^{6} [M^{-1}]	**A**
K[15]	association constant between NRII and PII	6.883×10^{10} [M^{-1}]	**S**
K[16]	association constant between NRII and GlnK	6.883×10^{10} [M^{-1}]	**S**
K[17]	association constant between NRII and ATP	1.759×10^{6} [M^{-1}]	**S**
K[18]	association constant between NRI and NRII-P	2.247×10^{11} [M^{-1}]	**S**
K[19]	association constant between NRI and acetlyphosphate	1.179×10^{5} [M^{-1}]	**S**
K[20]	association constant between NRII:PII and NRI-P	8.838×10^{10} [M^{-1}]	**S**
K[21]	association constant between NRI-P and NRII:GlnK	8.838×10^{10} [M^{-1}]	**S**
K[22]	association constant between PII, ADP and NRII-P	1.014×10^{9} [M^{-2}]	**S**
K[23]	association constant between NRII-P, ADP and GlnK	1.014×10^{9} [M^{-2}]	**S**
K[24]	association constant between NRI and Promoter(glnAp1)	4.158×10^{10} [M^{-1}]	**S**
K[25]	association constant between NRI and Promoter(glnLp)	1.840×10^{9} [M^{-1}]	**S**
K[26]	association constant between NRI-P and Enhancer(glnAp2)	1.166×10^{7} [M^{-4}]	**S**
K[27]	association constant between NRI-P and Enhancer(GlnK)	1.061×10^{5} [M^{-1}]	**S**
K[28]	association constant between NRI-P and Enhancer(nac)	1.643×10^{6} [M^{-1}]	**S**
km[1]	transcription rate constant for NRII	0.03 [min^{-1}]	**B**
km[2]	transcription rate constant for GS	0.03 [min^{-1}]	**B**

km[3]	transcription rate constant for NRI	0.03 [min^{-1}]	**B**
km[4]	transcription rate constant for GlnK	0.09 [min^{-1}]	**B**
km[5]	transcription rate constant for nac	0.03 [min^{-1}]	**B**
kmd[1-5]	mRNA degradation rate constant	0.12 [min^{-1}]	**B**
kp[1-5]	translation rate constant	20.0 [min^{-1}]	**B**
kpd[1-34]	protein degradation rate constant	0.035 [min^{-1}]	**B**
kx[1]	rate constant for dephosphorylation of NRI-P via PII	7.0 [min^{-1}]	**B**
kx[2]	rate constant for dephosphorylation of NRI-P via GlnK	7.0 [min^{-1}]	**B**
kx[3-4]	reaction rate constant	0 [min^{-1}]	**B**
kx[5]	rate constant for uridylylation of PII	7.0 [min^{-1}]	**B**
kx[6]	rate constant for uridylylation of GlnK	7.0 [min^{-1}]	**B**
kx[7]	rate constant for deuridylylation of PII-UMP	7.0 [min^{-1}]	**B**
kx[8]	rate constant for deuridylylation of GlnK-UMP	7.0 [min^{-1}]	**B**
kx[9]	rate constant for adenylylation of GS via GlnK	7.0 [min^{-1}]	**B**
kx[10]	rate constant for adenylylation of GS via PII	7.0 [min^{-1}]	**B**
kx[11]	rate constant for deadenylylation of GS-AMP via GlnK	7.0 [min^{-1}]	**B**
kx[12]	rate constant for deadenylylation of GS-AMP via PII	7.0 [min^{-1}]	**B**
kx[13]	rate constant for dephosphorylation of NRII-P	7.0 [min^{-1}]	**B**
kx[14]	rate constant for phosphorylation of NRI	7.0 [min^{-1}]	**B**
kx[15]	rate constant for phosphorylation of NRII	7.0 [min^{-1}]	**B**
kx[16]	rate constant for dephosphorylation of NRII-P via PII	7.0 [min^{-1}]	**B**
kx[17]	rate constant for dephosphorylation of NRII-P via GlnK	7.0 [min^{-1}]	**B**
Q[1]	synthesis rate of the glutamine (GS)	9×10^{9} [min^{-1}]	**E**
Q[2]	synthesis rate of the glutamate (GOGAT)	0.005×10^{9}[min^{-1}]	**E**
Q[3]	synthesis rate of the glutamate (GDH)	$(1/80)\times10^{9}$ [min^{-1}]	**E**
Q[4]	outflow and inflow rate of the 2-ketoglutarate (TCA)	1 [min^{-1}]	**E**
Q[5]	outflow rate of the glutamine	0.026 [min^{-1}]	**E**
Q[6]	outflow rate of the glutamate	0.220 [min^{-1}]	**E**
Q[7]	inflow rate of the 2-ketoglutarate	1.2 [min^{-1}]	**E**
Km[1]	Michaelis constant of glutamate (Q[1])	0.03 [M]	**E**
Km[2]	Michaelis constant of ammonia (Q[7])	0.03 [M]	**E**
Km[3]	Michaelis constant of glutamine (Q[2])	0.03 [M]	**E**
Km[4]	Michaelis constant of 2-ketoglutarate (Q[2])	0.03 [M]	**E**
Km[5]	Michaelis constant of 2-ketoglutarate (Q[3])	0.001 [M]	**E**
Km[6]	Michaelis constant of ammonia (Q[3])	0.03 [M]	**E**
Km[7]	Michaelis constant of Dummy (Q[3])	0.01 [M]	**E**
Km[8]	Michaelis constant of glutamine (Q[5])	0.03 [M]	**E**
Km[9]	Michaelis constant of glutamate (Q[6])	0.03 [M]	**E**
Km[10]	Michaelis constant of 2-ketoglutarate (Q[7])	0.03 [M]	**E**

References.

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