가중치를 가진 조절네트워크의 영향을 받는 *Escherichia coli*의 제약조건 대사모델

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The Constraint-Based Metabolic models of *Escherichia coli* under the influence of the weight-added regulatory networks

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서론

As the abundant experimental data on gene function and regulatory interactions have been accumulated in the field of molecular biology due to recent advances in high-throughput technology many data-driven approaches have become necessary to uncover functional inference and genetic regulatory networks from the large data sets. Though the traditional flux balance analysis has successfully predicted intracellular fluxes using stoichiometry, linear programming, and metabolic pathways, it has not automatically reflected any potential genetic effects in response to the environmental changes in the metabolic pathways. Thus in order to overcome the difficulties of the binary system, weight-added regulatory networks were devised so that the influence of sensors/regulators on target genes could be represented more flexibly. The weight-added regulatory networks consisting of many weight-added sub-networks were developed and incorporated into the FBA to remove inappropriate metabolic reactions generated by inconsistent regulatory events and to generate the dynamic level of expression of genes under the given conditions. Finally, in order to validate this integrated framework, the behavior of *E. coli* was simulated using specific environmental stimuli.

본론

Modeling and simulation software

LINGO (Lindo System Inc.) and LabView 7.1 (National Instruments, Inc.) were used to quantitatively calculate the optimal flux distribution, changes of substrate concentration and cell density, and expression patterns of genes over time..

Central metabolic network of *E. coli*

In this study, the central metabolic pathway of *E. coli* that produces all of metabolic by-products was chosen as a model system. This pathway consisted of 21 sensors/regulators, 19 operons, and 66 (55 %, 66/121) genes controlled by two or more regulators. Reconstructed weight-added regulatory networks contained 13 sensors/regulators (ArcA, CRP, DcuR, DcuS, FadR, FNR, FruR, GalR, GlpR, IclR, LacI, Mlc, and PdhR) that respond to O_2 (aerobic, anaerobic, and microaerophilic condition) and a variety of carbon sources (glucose, lactose, acetate, fructose, galactose, succinate, fumarate, malate, citrate, and glycerol).

Modeling the weight-added regulatory networks.

Generally, more than one genes occupy a metabolic pathway. Metabolic fluxes are controlled through these genes so that a cell has an optimal metabolic map that maximizes its growth rate in a given environments. *E. coli* is the best-understood single cell organism, and has the most abundant data in the sensing and regulation system.

Determining and calculating weights

Phosphotransferase systems (PTS) genes, *ptsHI-crr*, are the major system for transferring sugars, typically glucose, into the interior of *E. coli*. Regulatory proteins, such as CRP, FruR, and Mlc, have a direct influence on *ptsHI-crr* (Plumbridge, 1999, 2002; De Reuse and Danchin, 1988). Mlc and FruR act as repressors for *ptsG* and *ptsHI-crr*. A cAMP/CRP acts as an activator for *ptsHI-crr.*

Integrated framework for extending traditional FBA

Our integrated framework transformed the static analysis of the traditional FBA into a dynamic analysis in nature, as the reconstructed metabolic pathways were automatically modified by reflecting the genetic changes produced in response to any environmental condition. These genetic changes were represented in the reconstructed weight-added regulatory networks. The integrated framework consists of three parts: the FBA module, the weight-added regulatory network module, and the differential analyzer module.

결론

Model prediction using the integrated framework in aerobic batch culture with glucose and acetate reutilization

The weight-added regulatory networks directly and automatically determined the state of sensor/regulators and target genes without requiring an intervention in order to modify the reconstructed metabolic pathway into a specific metabolic pathway reflecting the regulatory effects of the given conditions. The initial parameters such as glucose, oxygen uptake rate, non-growth-associated maintenance value, and P/O ratio were obtained from the literature (Varma et al., 1994a; 1994b), and were used to simulate the *E. coli* behaviors.

Comparison between the predicted pattern of gene expression and the measured fluxes

The states and strength values of each gene were determined using the same procedures as given previously. Table 1 compares the simulated states and expression levels of genes and the in vivo flux distribution obtained from the literature (Schmidt et al., 1999).

Table . Comparison of the simulated expression levels of genes with in vivo flux distributions with aerobic and anaerobic growth in the presence of glucose.

Table 1. Comparison of the simulated expression levels of genes with in vivo flux distributions with aerobic and anaerobic growth in the presence of glucose.

Phase	*aceA*	*aceBK*	*aceEF-lpdA*	*acs*	*adhE*	*cydAB*	*cyoABCDE*	*dctA*	*dcuB*	*fumB*	*fdnGHI*	*focA-pflB*	*frdABCD*	*fumA*	*fumC*	*galETKM*	*glpACB*	*glpD*	*glpFK*	*lacZYA*	*mdh*
Aerobic Condition	*VS*	*VS*	*ST*	*VS*	*WK*	*IT*	*VS*	*VS*	*WK*	*ST*	*IT*	*VS*	*IT*	*VS*	*IT*	*IT*	*VS*	*ST*	*WK*	*VS*	*IT*
Fluxes	0	0	126.1	0	0	-	-	0	0	-	-	0	0	-	45.0	-	-	-	-	-	45.6
Anaerobic Condition	*VS*	*VS*	*ST*	*VS*	*VS*	*ST*	*VS*	*WK*	*VS*	*VS*	*IT*	*WK*	*ST*	*VS*	*IT*	*IT*	*IT*	*VS*	*WK*	*VS*	*IT*
Fluxes(Schmidt et al., 1999)	0	0	102	0	59.8	-	-	0	3.6	-	-	0	0	-	0.6	-	-	-	-	-	0.6

(Continued)	*mdh*	*ndh*	*ppsA*	*ptsG*	*ptsHI-crr*	*pykF*	*sdhCDAB-sucABCD*
	IT	*IT*	*ST*	*VS*	*ST*	*ST*	*IT*
	45.6		0	115	-	44.3	45.3
	IT	*IT*	*ST*	*VS*	*ST*	*ST*	*VS*
	0.6		0	105.3	-	67.0	4.3

감사의글

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