

Algebraic Method for the Analysis of Signaling Crosstalk

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Introduction

Signaling networks are complicated control systems comprised of a number of different molecules to activate or inactivate cellular mechanisms. Although kinetic simulation is one of the analytical approaches, it can be used only for the modeling of a small part, or of particular features, of living cells because it lacks both *in vivo* kinetic parameters and concentration profiles.

Methods

A constraints-based approach [1] requires the only information of stoichiometry of reactions and mass balances under the steady-state assumption, and it has been applied to the analysis of large-scale metabolic networks. However the number of enzymes in signaling networks cannot be assumed to be constant since an enzyme is often used as substrates or products of other reactions.

To obtain a minimal transduction pathway from input to output signals, we propose an enhanced modeling technique called as extreme signaling flow (ESF) that combines extreme pathways (EPs) obeying stoichiometric coefficients. ESF integrates enzyme activations and catalyzing reactions to represent as a coherent pathway. While an EP is a minimal unique unit characterizing a steady state, ESF is a minimal functional unit for signal transduction.

Results

The redundancy of networks was evaluated by the number of identical ESFs calculated from a network. High redundancy is indicative of a fault-tolerant property; low redundancy, on the other hand, indicates a high correlation between inputs and outputs. The analysis shows the numerous PKC-MAPK feedback routes and PP2A inactivation rather than CaMKII activation in LTP

induction, and the redundancy of PP1 activations in LTD.

Reaction participation analysis scores the ESFs occupancy in an objective reaction. A positive correlation indicates lethality and specific connectivity to stabilize cellular behavior. We calculated the scores of ESFs that contribute to induce LTP or LTD shown in Figure 1. The thickness of the lines in the figures reflects the proportion to scores. The scores of the exchange reaction of calcium in LTP and LTD were 99% and 78%, respectively. Reactions around the positive feedback loop involving PKC and MAPK had much higher scores in LTP than CaMKII. The reaction disassociating the complex of PP1 and phosphorylated I1 had 93% score in LTD. The activation of PLC_β induced by calcium stimulation had 88% score in LTP.

Table 1 compares the results of *in silico* knockout analysis with knockout mice experiments of the hippocampal CA1 region. The regulation of neuroplasticity was inferred from the number of ESFs suppressing the behavior by deletion of a targeting substance, resulting in the enhancement of LTP or LTD. 13 out of 16 experiments (81%) were identical. Four blank results (Ras, MAPK, AC and I1 in LTD) remain unknown *in vivo*, and many of our inferences were also no change in LTD but the knockout of I1 was inferred to lead the enhancement of LTD.

Concluding Remarks

We encountered some inconsistencies, particularly with respect to I1 and PKA. It needs to consider the difference of experimental conditions in constructing models. In addition our static model is not able to express the stimulation strength as the frequency of calcium oscillation. However, the proposed method certainly enables to analyze the robustness or fragility of large-scale signal transduction systems and to identify molecules that influence a whole system.

References

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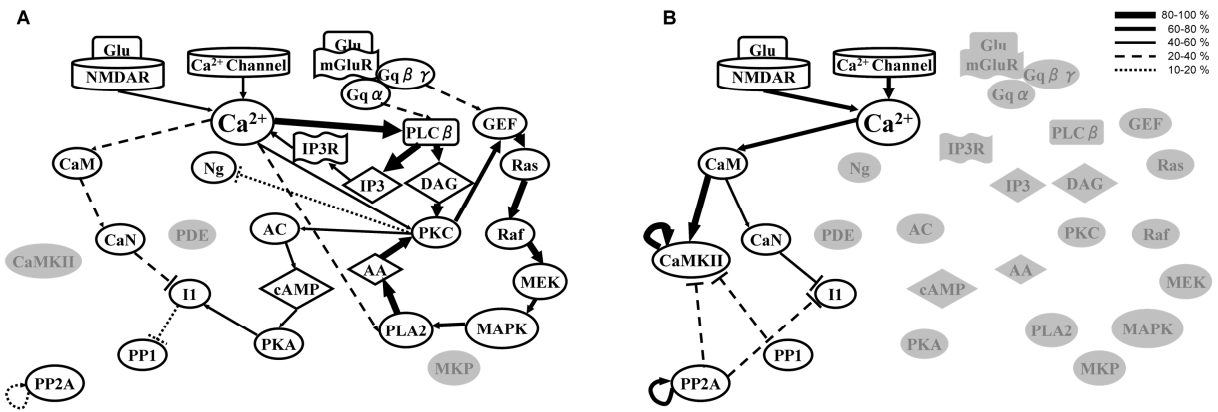


Figure 1: (A) Reaction participation analysis for LTP-inducing ESFs; (B) Reaction participation analysis for LTD-inducing ESFs. The line width reflects participation values.

Table 1: Comparison results of biological knockout mice and *in silico* knockout experiments. ↑ indicates up-regulation of LTP or LTD, ↓ down-regulation, and ⇔ no change, respectively.

	Knockout mice			In silico KO	
	LTP	LTD	Reference	LTP	LTD
PKC	↓	⇔	Abeliovich(1993)	↓	⇔
MAPK	↓		Winder(1999) Selcher(2003)	↓	⇔
mGluR	↓	⇔	Aiba(1994)	↓	⇔
CaMKII	↓	↓ ↑	Silva(1992) Steven(1994) etc	↓	↑
AC	↓		Wong(1998)	↓	⇔
PKA	↓	↓	Qi(1996) Abel(1997)	↓	⇔
CaN	↑ ⇔	↓ ⇔	Zeng(2001) Mallerent(2001)	↑	↓
Ng	↑ ↓	↓ ↑	Krucker(2002) Huang(2004)	↑	⇔
I1	⇔		Brown(2000)	↓	↑