506g Modeling of Heterotrimeric G-Protein Mediated Calcium Response in Raw 264.7 Macrophage Cells

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Abstract:

Calcium is an important secondary messenger. In macrophage cells (the cells that ingest bacteria as part of the immune response to infection), Calcium signaling is involved in the secretion of some of the cytokines and plays a role in phagocytosis (Kim and Sharma, 2004). Elevated Calcium levels have been observed in macrophages in HIV encephalopathy, a brain disease leading to injury to the central nervous system (Yi et al., 2004). Thus, quantitative study of Calcium signaling in macrophage cells is important. Recently the Alliance for Cellular Signaling (AfCS) has made available large amount of data on the dynamic response of free cytosolic Ca^{2+} upon stimulation with various stimulants (ligands) that activate a variety of receptors including G-protein coupled receptors (GPCR) and receptor tyrosine kinases (RTK) in RAW 264.7 macrophage cells. Data for gene-knockout of several key proteins such as G-beta, and data for stimulation by two ligands are also available. In the present work, a mathematical model is developed that can explain the Calcium response in RAW 264.7 cells, by utilizing mechanistic components from specific models for Calcium signaling in other cell types and from general models. The main challenges to be addressed are: (1) estimation of many parameters (reaction-rate constants, concentration of certain proteins that bind to Ca^{2+}, etc.) from experimental data on a single species (cytosolic free Ca^{2+}), and (2) variation in response in different cells (repeats) for the same dose of the stimulant (ligand).

The main mechanisms for the Calcium dynamics (Fink et al., 2000; Lemon et al., 2003; Marhl et al., 2000), including activation of phospholipase-C-beta (PLC-beta) and PLC-gamma, production of Inositol 1,4,5-trisphosphate (IP3) from phosphatidylinositol 4,5-biphosphate (PIP2) catalyzed by PLC-beta and PLC-gamma, IP3-induced release of Ca^{2+} from endoplasmic reticulum (ER) to cytosol, etc., are included in the model. Some details of receptor-activation (Yi et al., 2003) and GTPase-cycle are also included. This will help predict the response for gene-knockout of the relevant proteins. For most other mechanisms, simplified models presented in the literature are used. Model parameters were estimated by using a stochastic-search based nonlinear optimizer to minimize the fit-error between the experimental data and the model predictions. Except for Ca^{2+}, the initial conditions for other state variables are assumed or optimized to reflect that they can vary across different cells.

The model predicts Ca^{2+} response (both basal-level and the peak response) well for stimulation with single ligand C5a. Dose-response curve predicted by the model is also physiologically meaningful (sigmoid-shape) and the dose-level for half-maximum response (EC50 value) compares well with the EC50 value in other comparable systems. The stochastic-search based parameter estimator identifies several parameter-value sets with good fit to experimental data but with different dose-response curves. Data from several cells would be used to further constrain the parameters. Gene-knockout data would be used to validate the model.

Key words: Calcium, intracellular signaling, parameter-estimation, macrophage, Inositol 1,4,5-trisphosphate (IP3), C5a.

References:

Fink, C. C., B. Slepchenko, Moraru, II, J. Watras, J. C. Schaff and L. M. Loew, "An image-based model of calcium waves in differentiated neuroblastoma cells", *Biophysical Journal*, **79**(1), 163-183, 2000.

Kim, J. and R. P. Sharma, "Calcium-mediated activation of c-jun NH2-terminal kinase (JNK) and apoptosis in response to cadmium in murine macrophages", *Toxicological Sciences*, **81**(2), 518-527, 2004.
Lemon, G., W. G. Gibson and M. R. Bennett, "Metabotropic receptor activation, desensitization and sequestration-I: modelling calcium and inositol 1,4,5-trisphosphate dynamics following receptor activation", *Journal Of Theoretical Biology*, **223**(1), 93-111, 2003.
Marhl, M., T. Haberichter, M. Brumen and R. Heinrich, "Complex calcium oscillations and the role of mitochondria and cytosolic proteins", *Biosystems*, **57**(2), 75-86, 2000.
Yi, T. M., H. Kitano and M. I. Simon, "A quantitative characterization of the yeast heterotrimeric G protein cycle", *Proc Natl Acad Sci USA*, **100**(19), 10764-10769, 2003.
Yi, Y. J., C. H. Lee, Q. H. Liu, B. D. Freedman and R. G. Collman, "Chemokine receptor utilization and macrophage signaling by human immunodeficiency virus type 1 gp120: Implications for neuropathogenesis", *Journal Of Neurovirology*, **S10**, 91-96, 2004.

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