Creating synergism with a single allosteric enzyme

Finly Philip[1], Ganesh Kadamur[1,2], Rosa González Silos[1,3], Jimmy Woodson[1], and Elliott M. Ross[1,2]

***Short Abstract* — Strongly super-additive responses yield cellular outputs only when two signals are present, acting as coincidence detectors. The G protein subunits Gβγ and $G\alpha_q$ activate phospholipase C-β3 (PLC-β3) >10-fold more than additively, which accounts for synergistic Ca^{2+} transients observed during co-stimulation of G_i- and G_q-coupled receptors. A general two-state allosteric model quantitatively explains $G\alpha_q$-Gβγ synergism and why only PLC-β3 displays synergism. It predicts that any enzyme regulated by two allosteric activators will be a coincidence detector if its conformational equilibrium is strongly biased toward the inactive state in the absence of ligands, with maximal synergism being approximately proportional to this bias.**

***Keywords* — Synergism, phospholipase C-β, G protein signaling, two-state allosteric model, coincidence detection**

I. INTRODUCTION

CELLS use strong synergism between incoming signals to create coincidence detectors that produce a response only to simultaneous signals: a biochemical AND gate. Synergistic enzyme activation is poorly understood and is widely considered to be a complex phenomenon. In general, when will an enzyme that is stimulated by two or more ligands display a synergistic response? We conducted *in vitro* studies with purified phospholipase C-β isoforms to determine requirements for synergistic activation, and used mathematical analysis of a two-state model to generalize the requirements for any enzyme to be synergistically activated by two ligands. The model explains why of all the PLC-β isoforms, only PLC-β3 displays synergism. By further analyses of the model parameters, we were able to determine the minimal requirements for any enzyme to display synergism to two simultaneous inputs.

II. RESULTS AND DISCUSSION

A. $G\alpha_q$ and Gβγ activate only PLC-β3 super-additively

Purified PLC-β3 is activated by a combination of $G\alpha_q$ and Gβγ up to 10-fold more than the sum of the activities elicited by the two subunits added separately. Synergism exceeded 2-fold over relevant $G\alpha_q$ and Gβγ concentrations and was robust to changes in experimental conditions. $G\alpha_q$-Gβγ synergism on PLC-β3 appears to account for synergistic cellular responses to simultaneous stimulation of G_i and G_q coupled receptors. No other components are required. Other PLC-β isoforms did not display synergistic responses, consistent with *in vivo* data [1].

B. A general two-state allosteric model for synergistic enzyme activation

Synergistic activation of PLC-β3 can be described quantitatively by a simple and classical two-state allosteric model [2]. The model requires only that the enzyme exists in 2 states (inactive and active) and that both subunits bind to the active state preferentially. This model fits data for all the PLC-β isoforms.

C. The model predicts general requirements for synergistic response by a two-state enzyme

Quantitative examination of the two-state model indicates that any enzyme with sites for two allosteric activators can act as a synergistic coincidence detector if it strongly (>99%) favors the inactive state in the absence of ligands. Synergism is approximately inversely proportional to fractional basal activity. Greater bias for the inactive state produces greater synergism and makes synergism increasingly robust to variations in ligand concentration and ligand efficacy. PLC-β3 is a coincidence detector because its intrinsic basal activity is very low (0.1%). The model also explains why the other PLC-β isoforms do not display synergistic activation by G protein subunits - PLC-β2 has high basal activity (15%) while PLC-β1 has a very low intrinsic response to Gβγ.

III. CONCLUSION

The ability of an enzyme to display strongly synergistic activation depends on its having a strongly inhibited basal activity in the absence of stimulatory signals. This simple rule can lead to the discovery of new synergisms and cellular signaling crosstalk.

REFERENCES

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[1]Department of Pharmacology, University of Texas Southwestern Medical Center, Dallas, TX 75235

[2]Email: gkadam@mednet.swmed.edu, ross@utsw.swmed.edu

[3]Universidad de Valladolid, Departamento de Estadística e Investigación Operativa, Facultad de Ciencias, Valladolid, España