

GPCR signaling architecture in mammalian immune cells

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Introduction

Understanding the logic behind complex mammalian signaling networks is one of the research goals that have major medical implications. The “bow-tie” network structure has been proposed to play an important role in biological systems and may entail a small number of core molecular species constitutes ‘classifier hyperspace’ where the reactions to various inputs can be classified within *sub*-regions within the hyperspace that consists of activation levels of core elements of the bow-tie structure[1]. With regards to signaling network it has been reported that bow-tie structure also exists in epidermal growth factor receptor signaling, Toll-like signaling and GPCR signaling. In GPCR signaling network cAMP and Ca²⁺ dynamics can categorize ligands the cell is exposed and can predict groups of genes *up*-regulated by the stimuli. A publicly accessible B-Cell and Macrophage datasets from the Alliance of Cellular signaling [2] both has been used for this study [3,4].

Methods and results

Ligands were classified upon the elevation level of cAMP and Ca²⁺ based on the expertise provided with data. Ligands with increase of either molecule were assigned ‘YES’ for it, and ‘NO’ otherwise. Groups of ligands for both cells are depicted in Fig.1, and for example, YES/NO assessment for PGE2 means that it induces cAMP synthesis.

Next, unique *up*-regulated genes by one group of ligands and *down*-regulated by others at each time mark were selected, and positive cumulative expression (PCE) for each gene over a group has been calculated by formula below

$$PCE_t = \sum_{i=1}^n R_i$$

where $t=0.5$ and $t=1h$ are first time marks for B-Cell and macrophage, $n \geq i \geq 1$ is a number of ligands in each group, and R_i is an expression value of gene for the ligand. Then we calculated the PCE_{2h} and PCE_{4h} for each gene over 32 B-Cell ligands and 5 macrophage ligands with available microarray data to observe changes in gene expression after the termination of cAMP and Ca²⁺ activity, limited to first time marks. PCEs were clustered by KMC with Euclidean distance and complete linkage metrics.

For comparison we depict YES/NO and NO/NO groups in Fig.2.

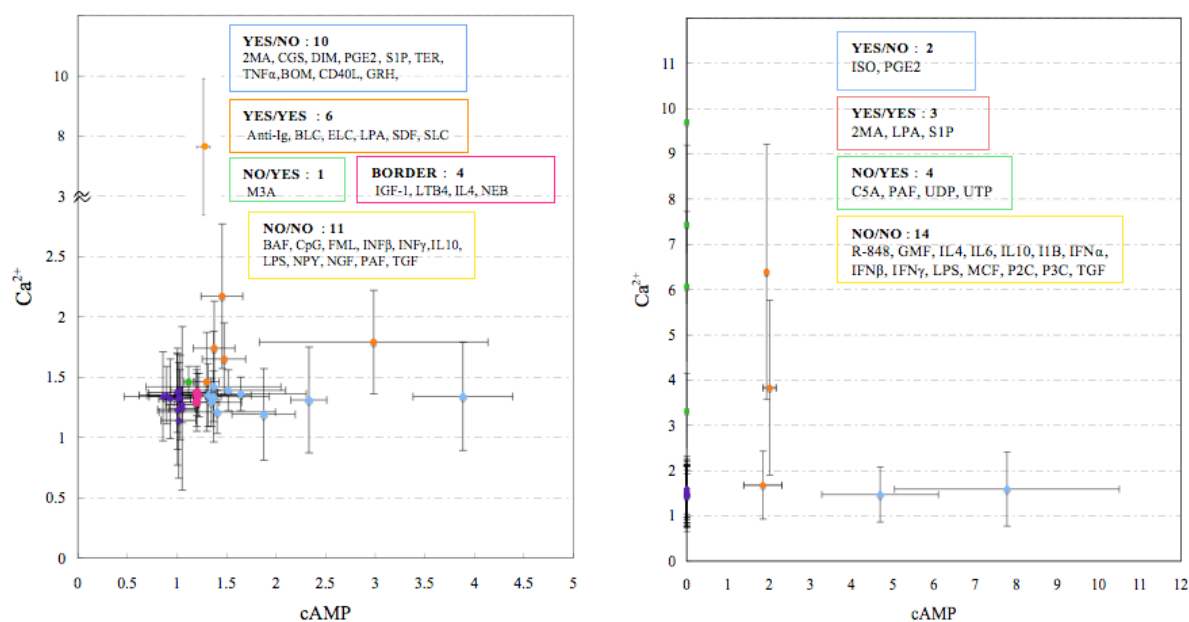


Figure 1. 32 ligands of B-Cell and 22 ligands of macrophage classified by fold change peaks into 5 and 4 groups, respectively. BORDER group includes ligands with intermediate fold changes irrelevant to any other ligands group.

In YES/NO clusters many genes are involved in transcription, ion, electron transport, enzymatic, receptor and GTPase activities. For macrophage Gs and Gq/11 coupled GPCRs have been identified: prostaglandin E receptor, fibroblast growth factor receptor, bombesin-like receptor, 5-hydroxytryptamine receptor, etc. For B-Cell 3 receptors with Gs binding activity and genes with non-GPCR receptor activity, such as B-cell receptor associated protein, MCH class 1 gene, CD80 antigen precursor, leukocyte receptor genes have been found.

NO/NO clusters included Tumor Necrosis Factor receptors, Toll-like receptors, Chemokine receptors and larger groups of apoptotic and immune response genes. Although transcription activation genes are many they belonged to *down*-regulated clusters in both cells (c14).

In YES/YES clusters (not shown) 7/9 receptors are Gs and Gi/o types GPCRs, contributing to feedback loop between intracellular cAMP and Ca²⁺[5].

We report about distinct functions of various *sub*-regions within the immune system together with their specific receptors and transcription factors, as well as about cross-cells conserved regulatory network.

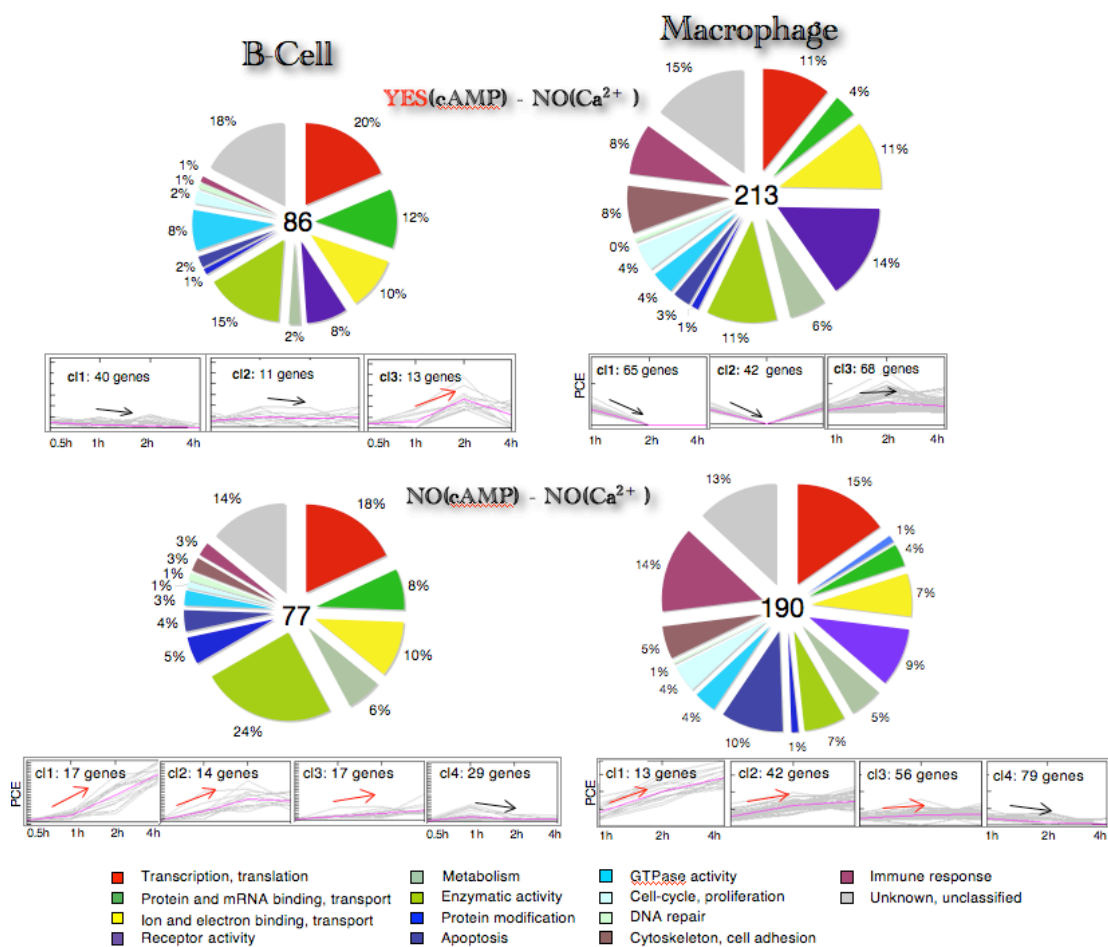


Figure 2. Functional representation and expression profiles comparison of YES/NO and NO/NO clusters of 2 cells. In clusters regulated by YES/NO ligands groups about 70% of genes loose their activity after 1 hour, however more than 60% of genes under NO/NO ligands groups are gradually *up*-regulated to 4 hours.

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