PROTEOMICS

Circuit maps for the cell

An assessment of the interactions between different receptor-mediated cellular signaling pathways may have revealed an unexpected degree of underlying compartmentalization and even simplicity in the cross-talk between these networks.

At a glance, even a single receptor's signal-transduction pathway can look intimidating, with its multiple inputs, outputs and regulatory checkpoints. That being the case, what sense can researchers possibly hope to make from the complex relationships between hundreds of different signaling pathways? Even in a simple binary scenario—imagine a row of light switches controlling a series of different lamps, where each switch-flip makes a room additively darker or lighter—more than a million different combinations can result from just a few dozen inputs. The reality is likely to be far messier, though, with outputs from one signaling pathway directly affecting the action of other receptors.

Messy or not, this is exactly the sort of question that the multi-institutional Alliance for Cellular Signaling (AfCS) came together to answer, and in a new article in *Nature Cell Biology*, University of Texas Southwestern Medical Center investigator Rama Ranganathan and his colleagues from the AfCS describe experiments in which they sought to characterize the cross-talk between the signaling processes initiated by 22 different receptor-ligand pairs—a total of 231 pairwise combinations.

They began by looking at the effects of each individual ligand on RAW 264.7 macrophages, quantifying outcomes that included calcium influx, protein phosphorylation and cytokine production. Having established these individual profiles, they proceeded with their pairwise stimulations and then compared the resulting outcomes against the responses that would be predicted to occur in the simplest 'additive' scenario. When the outcome differed significantly from this additive prediction, they assumed that there was a measure of cross-talk between the pathways being stimulated, resulting in a more complex input-output relationship.

Many of the data obtained were consistent with previously published findings about receptor signaling behavior, but Ranganathan and colleagues also were able to use this work to form new hypotheses about pathway interactions. For example, having observed consistent synergistic behavior resulting from costimulation with ligands that trigger calcium ion mobilization and ligands that trigger cAMP production, they were able to develop an experimentally testable model for this behavior and thereby identify a potential role for protein kinase A in mediating this interaction.

Each of the ligands studied interacted synergistically with at least one other ligand in the modulation of cytokine production—even though several of the ligands showed no independent effects on these processes. The authors found that they could organize their data into a relatively small number of clusters for which they observed similar synergistic behaviors between different types of ligands with regard to cytokine production. For example, costimulation of Toll-like receptors and G protein–coupled receptors consistently resulted in a particular pattern of production and suppression for the six different cytokines analyzed, whereas Toll-like receptor stimulation alone simply resulted in increased production for all six cytokines.

Ranganathan and colleagues postulate the existence of what they term 'interaction agents'—signaling circuits that specifically link together different types of ligand-receptor stimulatory pathways to generate particular synergistic outcomes—and suggest that identifying these interaction agents could prove essential to building a true understanding of cellular signaling processes. "Taken in pair-wise combinations, ligands begin to reveal their context-dependent roles in modulating final cellular outputs," the authors conclude. "Indeed, the data suggest that the primary activity of many input ligands is modulation of other signaling systems rather than direct control over cellular outputs."

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Natarajan, M. *et al.* A global analysis of cross-talk in a mammalian cellular signalling network. *Nat. Cell Biol.* **8**, 571–580 (2006).