Radio Transmission in the Cell

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***Short Abstract* — The canonical model of intracellular signal propagation involves the generation of secondary messengers by plasma membrane (PM) signaling complexes formed at ligand-activated receptors that communicate the signal intracellularly by diffusing to the nucleus, Golgi, endoplasmic reticulum (ER), etc. We describe a novel mode of intracellular information transfer – the "radio transmission" model – generated by the dual overlapping cycles that coordinate the activity (activation cycle) and dynamic relocalization (acylation cycle) of Ras [1]. Here, the constitutive acylation cycle plays the role of the "carrier wave", the varying level of Ras activity the "signal modulation", the PM and ER compartments the "transmitters", and the Golgi a passive "receiver".**

***Keywords* — Intracellular signaling, information transfer, ratiometric fluorescence microscopy, Ras signaling, Ras activation cycle, Ras acylation cycle, palmitoylation, network decoupling, network perturbation, compartmental modeling, radio transmission model**

I. Background

The small GTPase Ras plays an important role in many signaling networks in eukaryotic cells. Not surprisingly, specific mutants of Ras are potent oncogenes implicated in many cancer cell types. Ras activity in the cell is controlled at the compartmental level by GEFs and GAPs that mediate the exchange of GDP for GTP on Ras and vice versa. Additionally, irreversible farnesylation of Ras at the cysteine of its C-terminal CAAX box (followed by cleavage of the AAX residues) leads to an increase in affinity to membranes. A further increase in membrane affinity in some Ras isoforms arises from the reversible palmitoylation of one or more cysteines upstream of the CAAX box. Reversible palmitoylation has previously been shown by our group to lead to a dynamic, unidirectional, intercompartmental cycling of Ras known as the "acylation cycle" [2].

The coordination of Ras activity at distinct intracellular compartments is clearly a very important task of the cell, since active Ras is known to signal differently when present at distinct intracellular compartments. For example, in mammalian cells, Ras signaling from the PM leads to activation of the Erk pathway, whereas Ras signaling from the Golgi initiates the Ral and JNK pathways [3]. In fission yeast, PM-localized Ras supports mating but not changes in cell morphology, whereas the converse is true for ER-localized Ras [4]. We were interested in how distinct compartmental activities of Ras could be coordinated by the cell in the presence of its rapid and constitutive intercompartmental relocalization due to the acylation cycle.

II. Results

As the compartmental level of Ras activity is determined by its dual overlapping activation and acylation cycles, it was necessary to develop experimental approaches that could decouple these cycles. This was accomplished using both compartment-localized ("non-acylation-cycling") Ras isoforms and through interference with the global activity cycling of Ras (by microinjection of a GEF-sequestering mutant of Ras, RasS17N).

Quantitative ratiometric fluorescence microscopy in Madin Darby canine kidney (MDCK) cells using these and approaches revealed that the PM controlled early-time Ras activity and the ER controlled late-time Ras activity. Surprisingly, Ras activity was never controlled at the Golgi.

Compartmental modeling was performed in tandem with our experiments. A simple model based on exclusive control of Ras activity in the cell at the PM was sufficient to account for the initial pulse-like profile at the PM and its "echo" at the Golgi due to the acylation cycle; however, it could not account for late-time sustained activity at the Golgi long after activity at the PM had ceased. Inclusion of late-time Ras activation at the ER in the model accounted for this Golgi activity and motivated further experiments using ER-localized Ras that later confirmed the presence of this hypothesized late-time ER GEF activity.

III. Outlook

Reversible palmitoylation of Ras is present in all eukaryotic cells, where it presumably functions, at least in part, to rapidly communicate Ras activity between intracellular compartments. How eukaryotic cells utilize the unique properties of this ancient system to transfer intracellular information remains an open question.

References

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