Feedback regulates information transmission in a GPCR-MAPK signaling system

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***Short Abstract* — We developed *in vivo* microscope-based translocation and FRET reporters to quantify multiple signaling events during initial yeast pheromone response. The signal, which overshoots then declines to a plateau, takes about two minutes to propagate from the cell membrane to the nucleus. The signal decline results from MAP kinase-mediated feedback that acts on upstream membrane signaling events. The effect of this feedback is to maximize the amount and precision of information about external pheromone concentration transmitted to downstream cellular processes.**

I. Background

THE *Saccharomyces cerevisiae* pathway that measures extracellular mating pheromone and brings about the appropriate cellular responses is a prototype for eukaryotic G-protein coupled receptor-MAP kinase cascade signal transduction systems [1]. Very little is known about the quantitative dynamics of molecular events as signal is transmitted from the membrane to the nucleus during initial response to pheromone. Accordingly, little is known about how information about external pheromone concentration is encoded and transmitted by these molecular processes.

II. Summary of results

A. We created in vivo reporters to quantify membrane and nuclear signaling events.

We modified system proteins with fluorescent proteins to monitor, in single cells, upstream and downstream signaling events. To measure upstream signal, we quantified the recruitment of fluorescent protein-tagged MAP kinase scaffold Ste5 to pheromone-activated G-proteins in the membrane. To measure downstream signal, we designed a reporter of nuclear MAP kinase activity. We tagged the pheromone responsive transcription factor Ste12 and one of its inhibitors with fluorescent proteins. We then quantified downstream signal by measuring changes in pheromone-induced nuclear fluorescence-resonance energy transfer .

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B. We characterized baseline system physiology.

We mapped out signal dynamics at the membrane and nucleus using our newly developed reporters, and combined these with biochemical measurement of MAP kinase activation, which happens between Ste5 membrane recruitment and Ste12 de-repression in the nucleus, and mRNA levels of a pheromone-induced gene *FUS1,* which occurs downstream of Ste12 de-repression.

System output at each measured point peaks and declines toward a plateau. This peak takes about two minutes to propagate from the membrane to the nucleus. When the concentration of Ste5, a numerically limiting system component, is increased, the signal magnitude increases, yet signal propagation time does not. There is no desensitization at these early timescales; the signal magnitude in naive cells to high pheromone is the same as is cells pre-treated with low pheromone.

C. We identified a MAP kinase-mediated feedback that shapes the signal.

We found that the signal decline requires the kinase activity of the MAP kinase Fus3, and that this feedback acts upstream by reducing Ste5 membrane recruitment. This feedback–driven decline requires the regulator of G-protein signaling Sst2.

D. We found this feedback maximized information transmission by this system.

The effect of this feedback is to maintain an overlap of dose responses of upstream and downstream events. By doing so, the maximal range of upstream responses is represented in the downstream response curve, and any noise introduced at upstream events is not amplified as signal is transmitted. Thus, this feedback is a mechanism for tuning information transmission in this system.

III. Conclusion

The quantitative measurement of molecular events at relevant timescales reveal dynamics that improve both our mechanistic understanding of the system and how these mechanisms relate to fundamental biological processes such as information processing.

References

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