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Review

Cell signaling: What is the signal and what information does it carry?

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ABSTRACT

This paper reviews key findings from quantitative study of the yeast pheromone response system. Most come from single cell experiments that quantify molecular events the system uses to operate. After induction, signal propagation is relatively slow; peak activity takes minutes to reach the nucleus. At each measurement point along the transmission chain, signal rises, overshoots, peaks, and declines toward steady state. At at least one measurement point, this decline depends on negative feedback. The system senses and relays percent receptor occupancy, and one effect of the feedback is to maximize precision of this transmitted information. Over time, the system constantly adjusts quantitative behaviors to convey extracellular ligand concentration faithfully. These behaviors and mechanisms that control them are likely to be general for metazoan signaling systems.

1. Introduction

One central distinction between biological systems and complex dynamic non-living systems (from asteroid belts to hurricanes) is the centrality of information to the living state. Cells operate upon information about extracellular conditions, about internal processes, and upon information stored in their genome, to make decisions that determine their future actions. The continuing triumphal progress of the molecular biological agenda (begun in the 1930s [1]) had by the 1990s revealed much of the machinery that carries out these functions. One important next step is to understand more formally the relationship between these molecular components and the flows of information on which they operate. In order to find a relatively accessible entry point to this complex general issue, my coworkers and I have studied the quantitative function of a particular cell signaling system (here, a system that senses an extracellular condition, and transmits that information deeper into the cell).

Like all cell signaling systems, the yeast pheromone response system operates via chains of molecular events. The system operates in *Saccharomyces cerevisiae*, a model eukaryote. *S. cerevisiae* is a particularly tractable experimental organism; in particular, it features facile directed genetic manipulation, it is well suited for forward genetic experimentation, and it is supported by well-developed genomic and proteomic resources. Both specific molecular components of this system (e.g. seven helix G protein coupled receptors) and general themes (scaffold proteins, protein kinase cascades) are widely conserved throughout higher eukaryotic signaling systems.

Fig. 1 depicts some of the molecular details of the system and its operation. Pheromone binding to the Ste2 receptor causes dissociation of the single yeast heterotrimeric G-protein into a monomeric GTPase, Gpa1, and a dimer, Ste4–Ste18. Upon dissociation of the G-protein, Ste4 recruits the mitogen-activated protein kinase scaffold, Ste5 to the membrane. (Reassociation of Gpa1 with Ste4–Ste18 is promoted by the GTP-activating protein (GAP) function of the regulator of G-protein signaling (RGS-protein) Sst2 (not shown).) Ste5 recruitment leads to activation of the MAP kinase cascade, in which each of the protein kinases Ste20, Ste11, Ste7, and the MAP kinase Fus3 sequentially phosphorylates the next. Phosphorylated Fus3 translocates to the nucleus and phosphorylates Dig1 and Ste12, eliminating Dig1 repression of Ste12, a transcriptional activator. Ste12 activates transcription of pheromone responsive genes (shown in figure as PRGs, here gene derivative that fuses a pheromone-inducible promoter to YFP). Ribosomes translate mRNAs into proteins. In general, this signaling system is well understood, and its operation is well reviewed [2].

2. Single cell and other methods

When possible, cells are derived from a single reference strain, ACL 379 [3], which is in turn a descendent of the lab strain W303a. In particular, the strain carries a loss of function mutation in the *bar1* gene, so that it does not produce the secreted protease that degrades extracellular pheromone. In addition, most derivatives carry an inhibitor-sensitive allelic variant of the Cdc28 cell cycle

Abbreviations: FRET, fluorescence resonance energy transfer; RFP, red fluorescent protein; YFP, yellow fluorescent protein; MAP kinase, mitogen-activated protein kinase; DoRA, dose-response alignment; DoRR, dose-response relationship.

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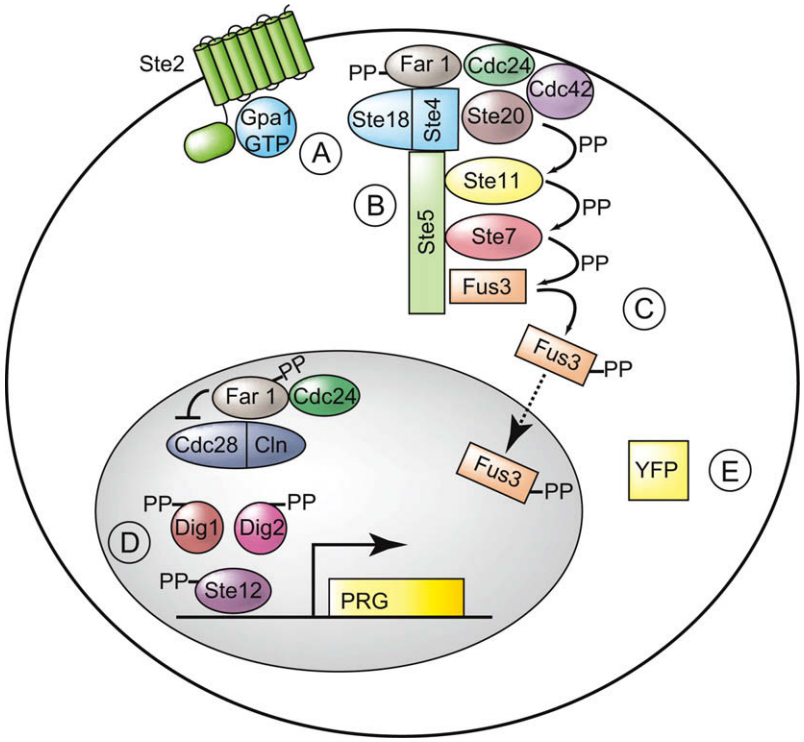

Fig. 1. Operation of the yeast pheromone response system. Proteins are indicated by labeled ovals, translocation by dotted lines, protein activation by arrows, inhibition by T-bar arrows, and protein association by double-headed dashed arrows, and measurement points by capital letters. Pheromone binding to the Ste2 receptor causes dissociation of the single yeast heterotrimeric G-protein into a monomeric GTPase, Gpa1, and a dimer, Ste4–Ste18 (measurement point A in figure). Reassociation of Gpa1 with Ste4–Ste18 is promoted by the GTP-activating protein (GAP) function of the regulator of G-protein signaling (RGS-protein) Sst2 (not shown). Upon dissociation of the G-protein, Ste4 recruits the MAP kinase scaffold, Ste5 to the membrane (point B in figure). Ste5 recruitment leads to activation of a protein kinase cascade, in which the proteins Ste20, Ste11, Ste7, and the MAP kinase Fus3 (and Kss1, not shown) sequentially phosphorylate one another. Phosphorylated Fus3 (point C in figure) translocates to the nucleus and phosphorylates Dig1 and Ste12, causing dissociation or conformational change in relationship between Dig1 and Ste12 (point D in figure), derepressing activation by Ste12, a transcriptional activator. Ste12 activates transcription of pheromone responsive genes (here, PRG); here, figure shows a gene derivative which fuses a pheromone-inducible promoter from the PRM1 gene to YFP. Transcribed mRNA is translated into protein by ribosomes (not shown) into yellow fluorescent protein (YFP, measurement point E).

protein kinase, so that investigators can disable cell cycle progression, and eliminate cell cycle dependent variation from any measurements.

Descendent strains also carry reporter constructs to enable quantification of different molecular events within the signaling pathway. By doctrine, when possible reporter constructions replace the native chromosomal gene copies with derivatives (such as fluorescent protein derivatives) expressed from the native promoters. By doctrine, we typically verify that the expression of fluorescent protein derivatives is equivalent to that of the native proteins by Western gels, comparing the amounts of expressed proteins to those of wild-type proteins determined from careful Western gel and fluorescent protein quantification [4]. Construction of most of the strains is described in the main text and supplemental materials of three papers, Colman-Lerner et al. [5], Gordon et al. [4], and Yu et al. [6].

These strains enabled single cell measurements of different molecular events the system uses to operate. We refer to molecular events for which we can measure system activity as "measurement points" (Fig. 1). These include the recruitment of yellow fluorescent protein- (YFP-) tagged Ste5 scaffold to the membrane [6] (Colman-Lerner et al., unpublished) (point B in figure), de-repression of YFP-tagged Ste12 and a cyan fluorescent protein- (CFP-) tagged derivative of the transcription suppressor Dig1 (measured by loss of fluorescence resonance energy transfer (FRET) between these chromophores) [6]) (D in figure), and expression of pheromone-inducible YFP and (red fluorescent protein) RFP reporter genes [4–6] (E in figure). We also used unrelated strains, W303 derivatives [7], that carried CFP and YFP G protein reporter constructs enabling single cell measurements of G protein dissociation (A in figure) by loss of FRET.

We induce system activity by single-step additions of pheromone to minimal medium [5,6]. We perform cell cytometry and quantification with epifluorescence microscopy, the open source software package Cell-ID [4], and analyze by canned routines written in PAW and R [4,8]. In cell populations, we measure the amount of phosphorylated and total Fus3 protein kinase by careful quantification using Western gels probed with antibodies that register total protein and antibodies that detect activated protein [6], and second antibodies conjugated to an infrared fluorophore. We measure the amount of pheromone-induced FUS1 mRNA using a specific DNA probe and nuclease protection [6].

3. Signal propagation

Fig. 2a shows the most typical system input regime. At the start of an experiment, we expose genetically identical cells to a given external concentration of mating pheromone. The extracellular medium contains casein to block adsorption of the pheromone to the inside of the plastic vessel, so that extracellular pheromone concentration and total system input remain constant thereafter.

Fig. 2b shows propagation of the signal in the first few minutes after system induction [6]. It graphs, on the *Y*-axis, the total percentage activity at four different measurement points, against time on the *X*-axis. The utility of this graphic representation is that it immediately suggests operational ways to define and quantify signal propagation and the signal. For example, we can define peak signal as 100% of the maximum activity at a given measurement