

Single-cell imaging of membrane recruitment of signaling scaffold: fractional receptor occupancy is sensed quantitatively seconds after addition of ligand.

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Extended abstract.

Cell fate decisions usually involve a precise and regulated response to extracellular stimuli. In haploid *S.cerevisiae* yeast cells, sex pheromones (α and α factor) activate a prototypic signal transduction pathway in that includes a seven transmembrane G-protein coupled receptor and a MAP kinase cascade¹. This pathway triggers a developmental switch that initiates mating behavior. Here we investigated the single-cell dynamics of the early, plasma membrane-associated signal propagation in response to α -factor. For that purpose, we measure pheromone-dependent recruitment to the plasma membrane of the scaffold protein Ste5 using microscope-based cytometry techniques² (Figure 1a). Relocalization is very fast and, at high concentrations of α -factor, the time to half maximum recruitment is approximately 5 seconds (Figure 1). Recruitment occurs in all cells, is non-polarized, and does not depend on the position of the cell in the cell cycle. Surprisingly, cells in the initial stages of budding (in which Cdk1 blocks the pheromone pathway) also exhibit Ste5 recruitment. Our results support the conclusion that Ste5 is not actively transported to the membrane, but rather uses a simple, diffusion-based mechanism to move to the plasma membrane.

We studied how signal is established and maintained after Ste5 reaches the membrane. We found that starting 10 seconds after pheromone addition, the fluorescence of YFP-Ste5 at the membrane becomes progressively punctate (Figure 1c), which our evidence suggests is caused by formation of dimers or higher-order oligomers of Ste5. Then, on average it takes 15 minutes for YFP-Ste5 molecules to distribute to the location of the future mating projection. We are investigating the dynamics and molecular nature of “dot” formation, which our data suggests are essential for signaling.

We found that pheromone dose information (percent occupied receptor) is transmitted precisely through the pathway (Figure 2 and poster and abstract by Richard Yu). For example, at doses of pheromone that result in 20% of the maximum receptor occupancy, Ste5 recruitment reaches 20% of its maximum. Quite unintuitively, Ste5 recruitment reaches these levels faster

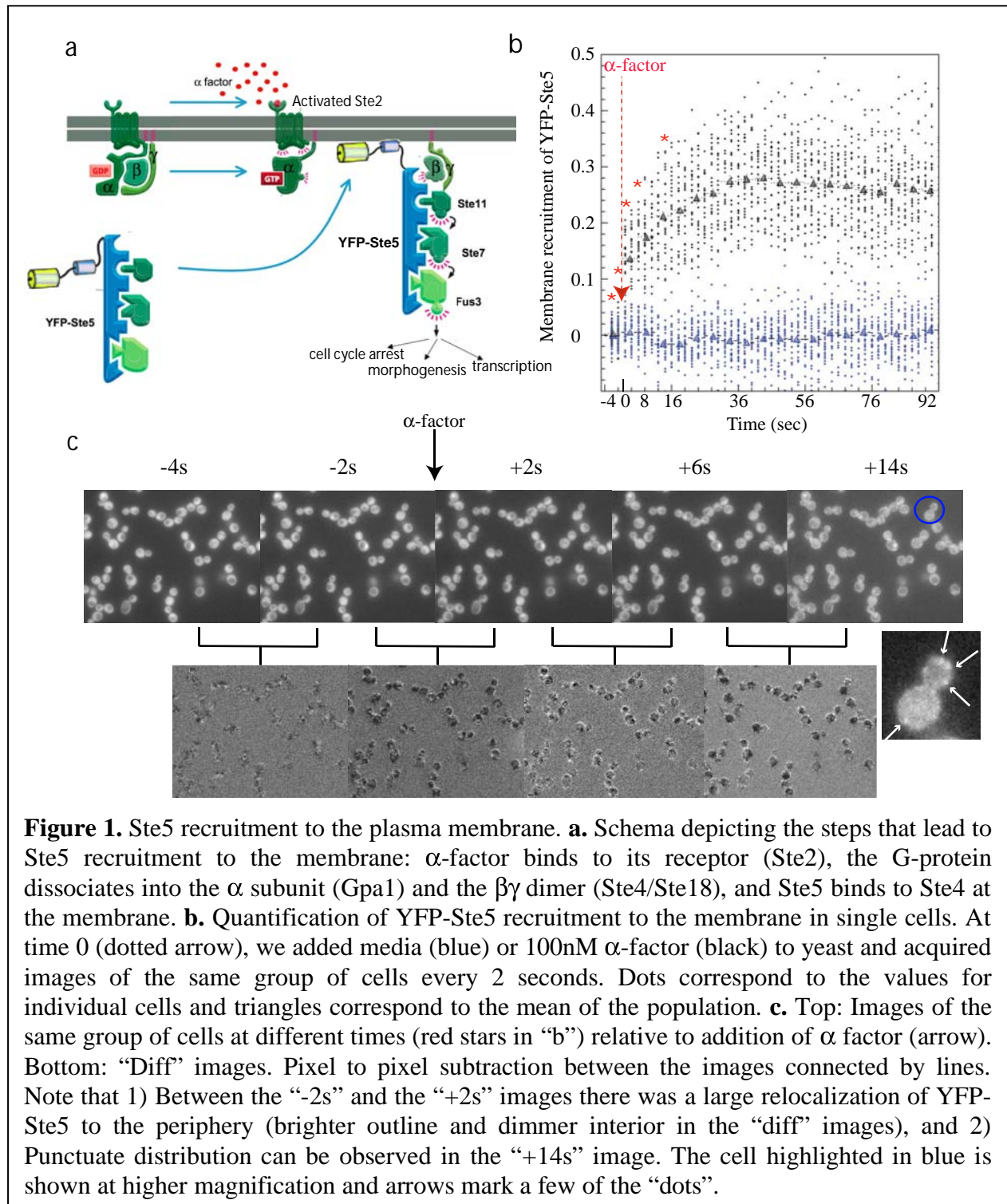
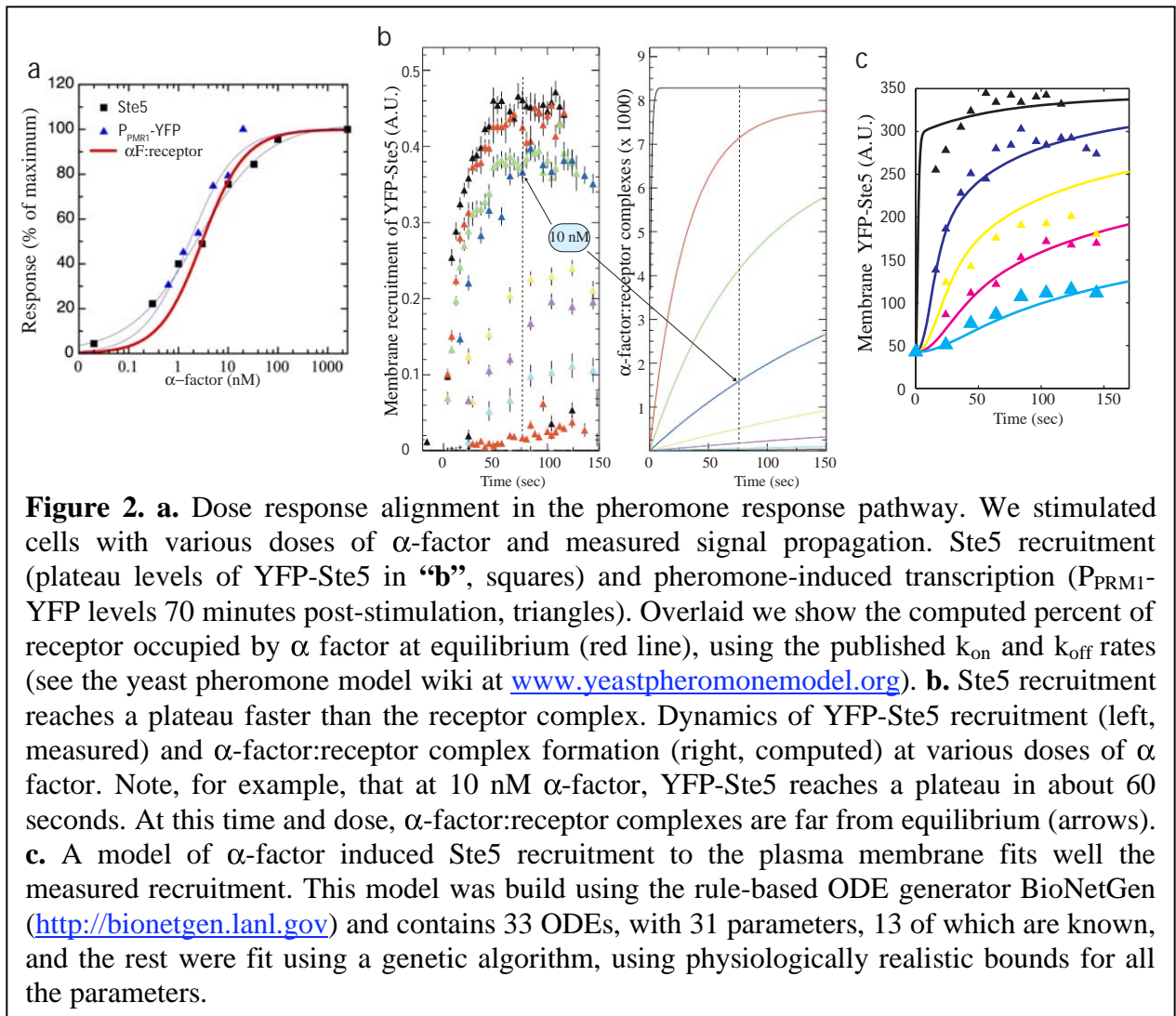


Figure 1. Ste5 recruitment to the plasma membrane. **a.** Schema depicting the steps that lead to Ste5 recruitment to the membrane: α -factor binds to its receptor (Ste2), the G-protein dissociates into the α subunit (Gpa1) and the $\beta\gamma$ dimer (Ste4/Ste18), and Ste5 binds to Ste4 at the membrane. **b.** Quantification of YFP-Ste5 recruitment to the membrane in single cells. At time 0 (dotted arrow), we added media (blue) or 100nM α -factor (black) to yeast and acquired images of the same group of cells every 2 seconds. Dots correspond to the values for individual cells and triangles correspond to the mean of the population. **c.** Top: Images of the same group of cells at different times (red stars in “b”) relative to addition of α factor (arrow). Bottom: “Diff” images. Pixel to pixel subtraction between the images connected by lines. Note that 1) Between the “-2s” and the “+2s” images there was a large relocalization of YFP-Ste5 to the periphery (brighter outline and dimmer interior in the “diff” images), and 2) Punctuate distribution can be observed in the “+14s” image. The cell highlighted in blue is shown at higher magnification and arrows mark a few of the “dots”.

than α -factor:receptor complexes are predicted to form (Figure 2b), suggesting that at least at the level of Ste5, the system may “measure” the rate of α -factor:receptor complex formation (“signal anticipation”). To address the source of signal anticipation, we developed two types of models. First, we developed a detailed model with standard chemical reactions describing the known biological mechanisms (pheromone binding, receptor internalization, G-protein



activation, Ste5 binding to G β γ and Ste5 dimerization). This model fits the data reasonably well (Figure 2c). Interestingly, the model predicts that that receptor endocytosis is not important for signal anticipation. In parallel, we are developing a simplified model with only four-species that captures pheromone-receptor binding, G-protein dissociation and Ste5 recruitment. Preliminary results suggest that a critical ingredient for signal anticipation is that the variables related to pheromone binding must evolve in time at a different time scale than those related to downstream reactions.

References

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