

Docking and scaffolding contribute differently to regulation of two overlapping yeast MAPK pathways

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All eukaryotes contain multiple mitogen-activated protein kinase (MAPK) pathways that regulate various cellular functions such as stem cell development, immune response, neuronal plasticity, cancer and arthritis. Furthermore, many of these pathways share one or more of their components. Due to their widespread involvement, it is critical to understand how MAPK pathways maintain specificity in signaling while sharing many of the components.

The budding yeast *Saccharomyces cerevisiae* is an ideal model to study MAPK signaling specificity because its mating and invasive (or filamentous) growth pathways share an entire MAPK cascade. The mating pathway MAPK cascade contains an upstream MAP kinase kinase kinase (MAPKKK) Ste11, MAP kinase kinase (MAPKK) Ste7, the MAP kinases (MAPKs) Fus3 and Kss1 and a scaffold protein Ste5 that binds the three proteins in the cascade. The invasive growth signal is also transduced through Ste11, Ste7 and Kss1. It has been proposed that Ste5, the scaffold for MAPK cascade, may provide specificity for the mating signal. MAPK cascade specificity is also maintained in part by MAPK docking sites, with a consensus motif (R/K)²⁻³ - (X)²⁻⁶ - L/I - X - L/I, on its upstream activators and/or downstream substrates. Two docking sites have been identified on Ste7 that are involved in its interaction with Fus3 or Kss1. Here we have looked at the role of Ste7 docking sites in combination with Ste5 scaffolding of Ste7 to understand signal transduction in both pathways.

We discovered that the two docking sites on Ste7 play a role in signal transduction in both the mating and invasive growth pathways. Mutation of either docking site (Ste7 2-19 Δ , Ste7 ds2) has some effect but a combination of mutation on both docking sites eliminates downstream signaling, including phosphorylation of the kinases Fus3 and Kss1. In contrast, Ste5 scaffolding of Ste7 is required for mating but not for invasive growth. Phosphorylation of Fus3 and mating signal are eliminated when Ste5 scaffolding is not available. Kss1 phosphorylation and invasive growth are unaffected in the absence of Ste7 scaffolding on Ste5. At the extreme eliminating Ste5 completely has an effect but does not eliminate the signal. We conclude that Ste5 contributes to mating and invasive growth but it is only crucial for mating. The docking sites on Ste7 are crucial for both mating and invasive growth.

We are using these data along with previously published results to make a model of the pathways using Ordinary Differential Equations (ODEs). Our approach is two-fold: first make a model that is robust enough to represent current interpretation of available data; use the model to make predictions about the role of negative feedback and ultrasensitivity in regulating specificity at a systemic level.