STE5 encodes a scaffold protein that assembles the protein kinases of the pheromone-activated MAPK cascade into an active complex during mating. Distinct regions of Ste5p interact with the MAPK Fus3p, the MAPK kinaseSte7p, and the MAPKK kinaseSte11p to form the active complex.Haploid yeast cells, which exist as either MATa or MATalpha mating types, initiate mating to form diploids through production of peptide pheromones. Pheromones bind to seven-transmembrane receptor proteins on cells of the opposite type: Ste2por Ste3p, leading to activation of the heterotrimeric G-protein composed of alpha subunit Gpa1p, beta subunit Ste4p, and gamma subunit Ste18p. Activation is mediated by exchange of GDP for GTP on the alpha subunit, causing it to dissociate from the heterotrimer. The resulting Ste4p-Ste18p dimer helps to recruit the scaffolding protein Ste5p and its associated MAP kinase cascade components, Ste11p, Ste7p, and Fus3p, to the membrane, where Ste11p is phosphorylated by the PAK kinase Ste20p; Ste11p then phosphorylates Ste7p, which phosphorylates MAPKs Fus3p and Kss1p. Both MAPKs phosphorylate the transcription activator Ste12p, which induces a number of mating-specific genes; in addition, Fus3p phosphorylates the cell cycle regulator Far1p, which mediates cell cycle arrest and is also involved, with Cdc24p, in polarized growth toward the mating partner.The Ste5p scaffold plays two positive roles in the mating signal transduction pathway. First, it binds the components of the MAPK cascade and holds them in an active complex; second, it associates with the plasma membrane, bringing the kinases to the plasma membrane where Ste11p can be activated by the Ste20p kinase. Membrane binding also promotes amplification of the signal, possibly by concentrating the bound kinases. Ste5p binds to Ste4p through the Ste5p amino terminal RING-H2 motif, which also mediates oligomerization of Ste5p. Oligomerization is important for proper signaling although it is not essential for mating. Although Ste4p helps recruit Ste5p and its associated MAP kinases to the membrane, membrane attachment is not absolutely dependent on interaction with Ste4p, as shown by the isolation of ste5 mutants that permit signaling in the absence of Gbeta-gamma. Rather, two regions within Ste5p are required for membrane association: a pleckstrin-homologydomainthat is conserved among Saccharomyces species and is essential for maximal pheromone signaling, and an amphipathic alpha-helical domain in the amino terminuscalled the PM/NLS domain.In vegetative cells Ste5p shuttles between the cytoplasm and nucleus, and the kinases are bound to it in the presence or absence of pheromone. Upon pheromone stimulation, a pool of nuclear Ste5p is exported to the plasma membrane and shmoo tip, colocalizing with the Ste4p-Ste18p complex of the G protein. Nuclear shuttling appears not to be essential for Ste5p translocation to the plasma membrane; thus it may serve as a mechanism for sequestering Ste5p to prevent inappropriate signaling. In addition to promoting efficient propagation of the pheromone signal, Ste5p also functions to downregulate signalingvia a negative feedback loop. Interaction of Fus3p with Ste5p stimulates autophosphorylation of Fus3p at one of two phosphorylation sites. Monophosphorylated Fus3p phosphorylates Ste5p, which leads to a decrease in signaling. Pheromone-induced cell cycle arrest is restricted to the G1 phase by G1 cyclin dependent protein kinases, which phosphorylate Ste5p at a cluster of CDK sites near the PM domain. This phosphorylation disrupts Ste5p membrane localization and therefore signaling. Ste5p thus acts as a point of integration for response to the external pheromone signal, which causes arrest in G1, and commitment to a new cell cycle, which inhibits pheromone signaling. While some other fungi contain Ste5p homologs, no obvious mammalian homologs have been identified. However, a number of proteins that function as MAPK cascade scaffolds have been identified in mammalian cells and other organisms.