CELL BIOLOGY

Signaling Crosstalk: Integrating Nutrient Availability and Sex

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In yeast, the mating response pathway is activated when a peptide pheromone binds to a heterotrimeric guanine nucleotide–binding protein (G protein)—coupled receptor, which leads to the activation of a mitogen-activated protein kinase signaling cascade and the stimulation of mating behavior. However, when nutrients in the environment are limiting, stimulation of the mating response would be maladaptive. A study indicates that the signaling pathways that respond to nutrient availability dampen the mating response by directly phosphorylating Gpa1, the G protein α subunit that initiates the mating response pathway. Snf1, the yeast homolog of adenosine monophosphate–activated protein kinase, is a highly conserved kinase that maintains energy homeostasis in response to nutrient limitation. The study found that the upstream kinases and phosphatase that control the activity of Snf1 also act on Gpa1 and provide a direct means to coordinate cell behavior and integrate the mating response with nutrient sensing.

Heterotrimeric guanine nucleotide–binding protein (G protein)—coupled receptors (GPCRs) constitute a large family of transmembrane proteins that function in signal transduction pathways that sense small molecules, hormones, and neurotransmitters and that mediate vision, olfaction, and taste. GPCRs are intensely studied, because as many as 40% of all pharmaceuticals target GCPRs. Structurally, GPCRs contain seven transmembrane–spanning helices, with the N terminus positioned externally, where it binds to a ligand, and the C terminus located internally, where it binds to a G protein (*1*). Ligand binding to the GPCR results in enhanced nucleotide exchange by the G protein α subunit, which results in guanosine triphosphate (GTP) binding, dissociation of the G protein α subunit from the βγ dimer, and the activation of downstream signaling. One of the most studied GPCRs is the Ste2 protein of *Saccharomyces cerevisiae*, which is the receptor for the mating pheromone known as α factor, and initiates the mating response and fusion to cells of the opposite mating type. Years of study have delineated the components of the Ste2 signaling pathway in yeast and have provided details about the mechanisms by which G proteins can activate mitogen-activated protein kinase (MAPK) signaling cascades. Taking our understanding to a higher level will require the elucidation of mechanisms by which the coordinated responses to different stimuli are integrated. Work by Dohlman and colleagues now describes how Ste2 signaling is dampened in response to nutrient limitation (*2*).

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Yeast cells live in an environment that fluctuates wildly between having nutrient abundance and scarcity. As a result, yeast cells have developed multiple signaling pathways that respond to the availability of sugars, nitrogen, amino acids, and other nutrients. The presence of glucose initiates multiple signaling pathways that converge on adenylate cyclase and protein kinase A (PKA). The primary activator of PKA appears to be the yeast homolog of the Ras protein (*3*); however, yeast also use a distinct GPCR, the Gpr1 protein, as a glucose sensor to activate adenylate cyclase and PKA. When glucose is plentiful, PKA phosphorylates many downstream targets that promote ribosome biogenesis and growth through the fermentation of glucose. When glucose is limiting, however, yeast activate an opposing signaling pathway that promotes energy conservation, ATP homeostasis, and aerobic growth. Signaling in response to glucose limitation is mediated by the kinase Snf1, the yeast homolog of adenosine monophosphate–activated protein kinase (AMPK). The exact mechanism that leads to the activation of Snf1 is not fully understood, but it probably involves sensing the cellular energy charge through direct binding to adenylate ligands, such as adenosine diphosphate (*4*). Snf1 is activated by phosphorylation on a conserved threonine residue in its activation loop. The phosphorylation status of Snf1 is controlled by the relative activities of the Snf1-activating kinases Sak1, Tos3, and Elm1 and the inactivating PP1 phosphatase, which is composed of the catalytic subunit Glc7 and the regulatory subunit Reg1. Clement *et al*. describe the molecular mechanism by which the Snf1-dependent pathway that senses limiting amounts of glucose acts on the mating pathway to reduce mating efficiency during times of nutrient stress. The mechanism by which one signaling pathway regulates a second provides insight into how cells integrate multiple stimuli to produce a coordinated response.

The mating pathway in yeast is initiated by the external binding of the pheromone α factor to the GPCR Ste2 (Fig. 1). Changes in the conformation of the ligand-bound Ste2 protein are transduced across the cell membrane to the cytoplasmic domain, which leads to nucleotide exchange and dissociation of the trimeric G protein complex composed of Gpa1, Ste4, and Ste18, which are homologs of the α, β, and γ subunits of mammalian G proteins, respectively (*5*). The Ste4-Ste18 (βγ) dimer activates Ste20, one of the prototypes of the p21-activated protein kinase family. Ste20 in turn directly activates the MAPK cascade that ultimately controls mating behavior. Clement *et al*. noticed that Gpa1 underwent cell cycle–dependent phosphorylation and that the responsible kinase was Elm1 (*6*). Elm1 localizes to the bud neck, where it activates other kinases that regulate septum formation and the spindle position checkpoint. Elm1 was also identified as one of three kinases capable of activating Snf1 (*7*). Additional studies of the Snf1 pathway suggest that the primary activator of Snf1 in response to nutrient stress is Snf1-activating kinase 1 (Sak1); however, in cells lacking Sak1, the functionally redundant kinases Tos3 and Elm1 mediate Snf1 activation. In their study, Clement *et al*. showed that the phosphorylation of Gpa1 protein increased in response to nutrient limitation and that Sak1 was the primary kinase responsible for this modification. They also showed that the Glc7-Reg1 complex, the same phosphatase that acts on components in the Snf1 pathway, dephosphorylated Gpa1. Thus, the activating kinases and inactivating phosphatase in the nutrient signaling pathway also regulate the phosphorylation of Gpa1. Functionally, Gpa1 phosphorylation correlated with reduced activation of the mating pathway MAPK signaling cascade, reduced activation of mating-specific gene transcription, and reduced formation of mating projections colorfully known in the yeast world as "shmoos."

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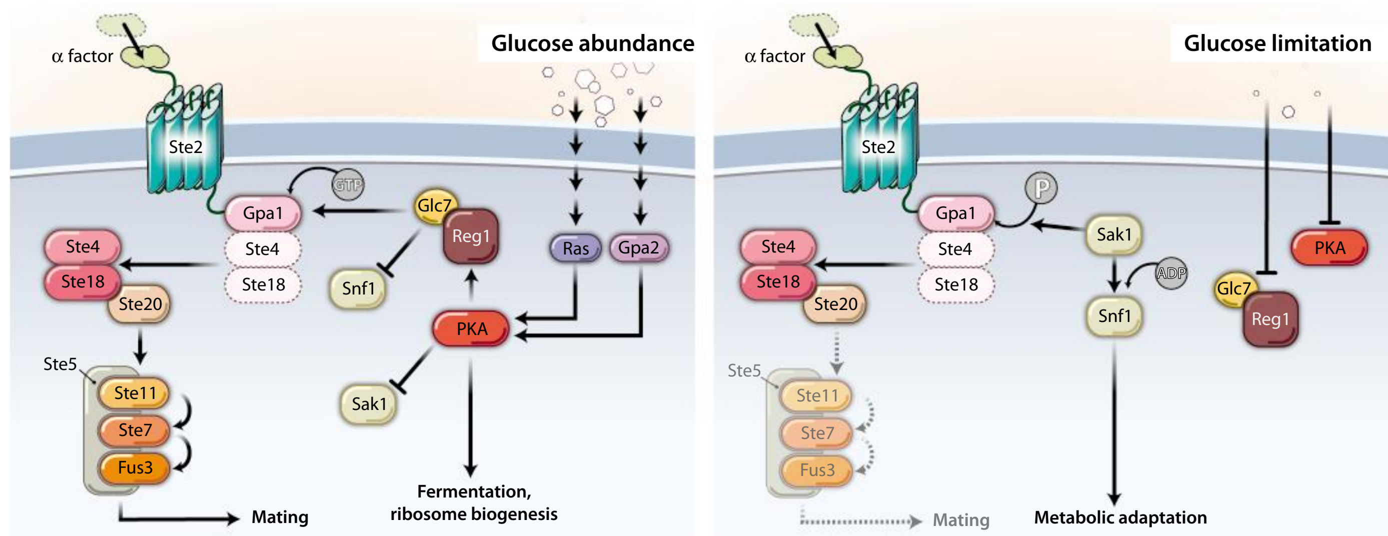

Fig. 1. Crosstalk between the signaling pathways that mediate nutrient sensing and the mating response. The mating response pathway is initiated when the pheromone α factor binds to the GPCR Ste2 at the plasma membrane. Ligand binding stimulates the exchange of GDP for GTP by Gpa1 and the dissociation of Gpa1 from the Ste4-Ste18 dimer of the G protein. Ste4-Ste18 interacts with Ste20 to stimulate the MAPK signaling cascade, culminating in the activation of Fus3 and the mating response. During conditions of glucose abundance, the Ras and Gpa2 pathways activate PKA, which promotes the Glc7-Reg1–dependent dephosphorylation of Gpa1 to maintain an efficient mating response. However, when glucose concentrations are limiting, PKA and the Glc7-Reg1 phosphatase complex are inactivated. Thus, the kinase Sak1 phosphorylates both Snf1 (the AMPK homolog), to initiate metabolic adaptation, and the Gpa1 protein, to reduce mating efficiency. The exact consequences of Gpa1 phosphorylation on G protein signaling remain to be determined.

Although this study provides strong evidence of an inverse correlation between Gpa1 phosphorylation and mating efficiency, a number of questions need to be resolved before we have a complete understanding of how nutrient limitation dampens the mating response. First, how does phosphorylation of Gpa1 affect its signaling potential? Does the phosphorylation of Gpa1 affect its nucleotide exchange or GTP hydrolysis capabilities? Does it affect dissociation of the trimeric G protein complex? Are the activities of other proteins in this pathway regulated by phosphorylation? These mechanistic questions have yet to be answered. Second, the upstream events that control nutrient-dependent signaling pathways are far from settled. Initial studies of the Snf1-activating kinases Sak1, Tos3, and Elm1 indicated that these kinases were constitutively active and not regulated in response to glucose availability (*8*); however, a study from Barrett *et al*. showed that mutations in components of the PKA signaling pathway lead to inhibition of Snf1 activity and that PKA directly modifies Sak1 (*9*). Additionally, the phosphatase activity of Glc7 appears to respond directly to nutrient availability. Studies that measured the phosphatase activity of Glc7 isolated as immune complexes showed rapid increases in activity in response to the addition of glucose to cell cultures. The increased activity of Glc7 was stable to purification, consistent with a posttranslational modification, and was dependent on PKA (*10*). Both of these studies indicate that PKA is playing an active role in turning off the Snf1 pathway by inhibiting Sak1 and activating Glc7-Reg1. Thus, mating efficiency may be highest when glucose is abundant and the effects of PKA on Sak1 and Glc7-Reg1 maintain Gpa1 in an unphosphorylated state. When nutrients are limiting, survival of the cell population may be maximized by reducing mating efficiency. In this way, some cells mate and thereby acquire additional genetic potential for survival in lean times, whereas other members of the population divert their limited resources toward metabolic reprogramming.

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