Heat Shock Analysis of the Interdependency of Pheromone and Glucose Sensing G-Protein Coupled Receptor Pathways in Saccharomyces cerevisiae

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Abstract
In Saccharomyces cerevisiae, the G-protein coupled receptors (GPCR) Gpr1p and Ste2p/Ste3p are known to initiate signaling through two separate pathways, the glucose sensing pathway and pheromone sensing pathway, respectively. One known role of the glucose-sensing pathway is to cause the cell to exhibit an increased sensitivity to heat shock when introduced to high concentrations of glucose after a period of starvation. The pheromone-sensing pathway does not share this characteristic. However, research in our lab has indicated that disruption of the Gpr1 pathway negatively affects mating in Mata cells. In an attempt to determine if the reciprocal effect might be present, mutants of Matα and Mata cells lacking components of the pheromone pathway were assayed for their sensitivity to heat. The results show that Mata cells exhibit no increased resistance to heat shock when the components of the pheromone-sensing pathway are removed, because the relative survival of cells after heat shock is approximately equal to the relative survival of wild type cells. On the other hand, Mata cells can exhibit a 10-100-fold increase in relative survival or resistance to heat shock when components such as Ste2, Ste4, Ste5, Ste7, STE11, STE18, and Fus3 are removed from the pheromone-sensing pathway. These results support the hypothesis that in Mata cells (but not Mata cells) there is a connection between the components of the pheromone and glucose sensing pathways that directly affects the cell’s response to heat shock in the presence of glucose. The interaction point between the two pathways is certainly crucial in regard to the heat shock stress response and, thus, is likely to play an important role controlling the many additional functions that each GPCR pathway executes.

OBJECTIVE
In Saccharomyces cerevisiae, the pheromone and glucose sensing pathways are two signal transduction pathways that use the G-protein receptors Ste2p/Ste3p and Gpr1p to regulate functions like mating and cell cycle arrest for the pheromone pathway and pseudohyphal differentiation, heat shock sensitivity, and growth inhibition for the glucose pathway. We have observed that the pheromone pathway is dependent on components of the glucose-sensing pathway and this study was to determine if the reciprocal effects are also present.

RESULTS
By comparing the survival rates before and after glucose addition for cell harboring different mutations, the effect that mutation of the pheromone sensing pathway has on activation of the glucose sensing pathway can be determined. Since the expected response of glucose addition is increased sensitivity to heat shock, cells exhibiting a higher relative survival than WT cells after heat shock can be inferred to play a role in the functionality of the Gpr1p (glucose sensing) pathway. Based on the shock analysis of BY4742 (Mata) cells, deletion of many components of the pheromone sensing pathway seems to have no effect on sensitivity to heat shock, and interaction between pathway components is not evident or at least insignificant in signaling the heat shock response. Conversely, the shock analysis of BY4741 (Mata) cells depicts increased resistance to heat shock when components including Ste2p (receptor), Ste4p (Gh), Ste5p (scaffold) Ste7p (MAPK), Ste11p (MAPK), Ste18p (G), and Fus3p are removed. While many components of the pheromone sensing pathway appear to have direct relation to the functionality of the heat shock response, the BGS protein Ste2p appears to play little to no role in the execution of the heat shock response in both Mata and Mata cells.

METHODOLOGY
BY4741(Mata) and BY4742(Mata) cells with various mutations of the pheromone pathway components were starved of glucose and partially depleted of glycerol when grown for 48h at 30°C. Each sample had one portion diluted and subject to a heat shock at 52°C for 15 minutes, while another portion was incubated with 4% glucose solution prior to heat shock. The solutions were then plated on YPD and information on the percent survival of cells was determined by colony counting.

CONCLUSION
The results indicate that interaction between the glucose sensing pathway and pheromone sensing pathway is present in Mata cells, but not Mata cells. In Mata cells, the successful execution of a heat shock response is dependent on the presence of Ste2p, Ste4p, Ste5p, Ste7p, Ste11p, Ste18p, and Fus3p, and the interaction point between the pathways can be inferred to lie downstream of the components (mutations) tested. Determining an interaction and the point of that interaction between pathways is worth exploring, because it can be an important tool in learning to regulate the cellular responses of one G-protein’s pathway by manipulating the common associated pathway.

Figure 1- Visualization of the/Estimated Sensory Pathway in Budding Yeast
* Pathways are courtesy of QIAGEN Sample & Assay Technologies

Figure 1- Heat Shock Analysis of BY4742 Ste11p, Ste2p, Ste3p, Ste4p, and Ste7p Mutants

Figure 2- Heat Shock Analysis of BY4741 Ste2p, Ste7p, Sst2p, and Fus3p Mutants

Figure 3- Heat Shock Analysis of BY4741 Ste4p, Ste18p, Ste5p, and Ste11p Mutants

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