Functional phosphoproteome analysis of yeast pheromone response system reveals loci of feedforward and feedback control.

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We have elucidated early phosphorylation-dependent system connections in a well-studied signal transduction system in *Saccharomyces cerevisiae,* the pheromone response system. We used a dual affinity purification strategy that included a metal affinity chromatography purification scheme to enrich for proteins that were modified with phosphate groups, and identified sites of phosphorylation for proteins in the pathway using protein mass spectrometry. We are particularly interested in phosphorylation events that might be important for system function or regulation, thus we focused on phosphorylation events that changed with time at early points after system induction. Our results suggest that a combination of comprehensive protein mass spectrometry, relatively simple genetic manipulation, and single cell readout, might allow identification and assignment of function to all the significant regulatory connections among the proteins in this particular signal transduction system. They further provide grounds to hope that, for certain systems in yeast and other prototype organisms, these methods can be extended to identify and understand the function of regulatory protein modifications.