Kinase-substrate analysis via diaPASEF phosphoproteomics

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The S. cerevisiae (yeast) ISR1 gene encodes a putative kinase with no specific function ascribed, however over expression of ISR1 is lethal. To determine the function and substrates of this kinase, we conducted diaPASEF phosphoproteomics experiment in both ISR1 knockout and ISR1 expressing cells. From this analysis we quantified 8,879 phosphopeptides. Of these sites, we identify 4 sites of phosphorylation on Gfa1, one of which (S332) was up-regulated by nearly 200-fold in the presence if the ISR1 kinase. Alanine mutations of this residue, as well as adjacent S/T residues, rescued the lethality associated with ISR1 over expression. These results suggest that Gfa1 is a bonafide substrate of ISR1, and implicates a role for ISR1 in the hexosamine biosynthesis pathway. In short, we demonstrate the utility of diaPASEF phosphoproteomics to rapidly and confidently determine the substrates and function for uncharacterized kinases.