Coupling auxin-inducible degradation with quantitative phosphoproteomics reveals a new role for $PP2A^{Rts1}$ in stabilizing eisosomes during mitosis.

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Protein functional characterization typically involves gene deletions, transcript knockdowns, or conditional mutations that can result in indirect physiological changes from prolonged target protein loss. This is problematic for many proteomic applications, like identification of kinase or phosphatase substrates. Chemical inhibitors are ideal, providing rapid and specific target inactivation. Unfortunately, specific chemical inhibitors are not available for most proteins. Inducible protein degradation technologies like the auxin-inducible degradation (AID) system are an attractive alternative, providing rapid depletion of virtually any target protein under diverse physiological conditions. AID acts as a surrogate for specific chemical inhibition, which minimizes non-specific effects associated with long-term target perturbation. Our lab has been interested in combining AID with proteomic methods to study signaling pathways and characterize direct kinase and phosphatase substrates. We established and validated a workflow for AID-coupled phosphoproteomics in budding yeast by targeting subunits of the abundant protein phosphatase 2A (PP2A). The AID system reduces endogenous levels of individual PP2A components by >85% within 10 minutes, leading to quantifiable phosphoproteomic perturbations by 20 minutes. Using this system, we demonstrate-PP2A in complex with its B-subunit Rox Three Suppressor 1 ($PP2A^{Rts1}$) contributes to the phosphoregulation of a conserved fungal-specific membrane protein complex called the eisosome. Eisosomes are attractive targets for novel antifungal therapeutics and play multiple physiological roles within the cell, including maintaining polarized growth. Rapid degradation of $PP2A^{Rts1}$ leads to significant increases in phosphorylation of multiple eisosome proteins, including the eisosome core protein Pil1. Fluorescence microscopy analysis of $PP2A^{Rts1}$-depleted cells showed a significant difference in Pil1-EGFP signal at the plasma membrane, consistent with evidence that eisosome phosphorylation leads to disassembly. We are currently testing the model that $PP2A^{Rts1}$ is required to maintain functional, hypophosphorylated eisosomes during mitosis, when cellular kinase activity peaks and many phosphatases are suppressed.