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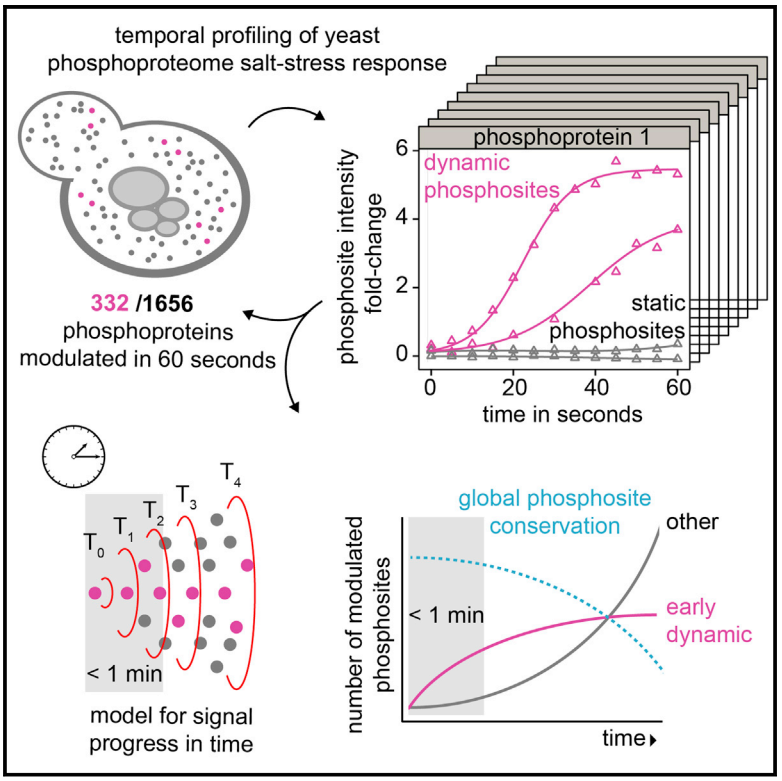
A Cell-Signaling Network Temporally Resolves Specific versus Promiscuous Phosphorylation

Graphical Abstract



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In Brief

Kanshin et al. report that conserved and putatively functional kinase- or phosphatase-substrate interactions in the high-osmolarity glycerol (HOG) response occur more rapidly than promiscuous interactions. They provide a rich data set of dynamic phosphosites that may be implicated in the regulation of the cell cycle, cytoskeletal dynamics, and morphogenesis.

Highlights

- A strategy for phosphoproteomics at high temporal resolution and coverage
- The HOG-signaling network involves 25% of the kinome and 10% of phosphatases
- Changes in functional phosphorylation occur more rapidly than promiscuous events
- Many potentially regulatory phosphosites in cytoskeletal proteins are reported

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A Cell-Signaling Network Temporally Resolves Specific versus Promiscuous Phosphorylation

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SUMMARY

If specific and functional kinase- or phosphatase-substrate interactions are optimized for binding compared to promiscuous interactions, then changes in phosphorylation should occur faster on functional versus promiscuous substrates. To test this hypothesis, we designed a high temporal resolution global phosphoproteomics protocol to study the high-osmolarity glycerol (HOG) response in the budding yeast *Saccharomyces cerevisiae*. The method provides accurate, stimulus-specific measurement of phosphoproteome changes, quantitative analysis of phosphodynamics at sub-minute temporal resolution, and detection of more phosphosites. Rates of evolution of dynamic phosphosites were comparable to those of known functional phosphosites and significantly lower than static or longer-time-frame dynamic phosphosites. Kinetic profile analyses indicated that putatively functional kinase- or phosphatase-substrate interactions occur more rapidly, within 60 s, than promiscuous interactions. Finally, we report many changes in phosphorylation of proteins implicated in cytoskeletal and mitotic spindle dynamics that may underlie regulation of cell cycle and morphogenesis.

INTRODUCTION

Signaling networks have evolved to respond to distinct environmental stimuli with coherent and specific responses. Yet recent evidence suggests that protein kinases, the major signaling enzymes in the cell, form a highly connected and perhaps irreducibly complex network (Bodenmiller et al., 2010; Breitkreutz et al., 2010). We have argued that complexity of the kinase network may have evolved to integrate and compare multiple signals, resulting in main and complementary responses to stimuli (Levy et al., 2010). Such a network could also generate promiscuous phosphorylation that has no functional consequence but confounds our understanding of the signaling response (Figure 1A; Landry et al., 2009; Levy et al., 2012).

It is generally viewed that signaling networks have evolved so that kinase- or phosphatase-substrate interactions are made most efficient by optimization of enzyme binding and specificity for substrate recognition sequences and structural organization through domain, adaptor, or scaffold protein binding (Bhattacharyya et al., 2006; Scott and Pawson, 2009; Zheng et al., 2013). We hypothesize that functional phosphorylation of specific substrates occurs rapidly following stimulation but that promiscuous phosphorylation takes place more slowly, following random encounters with neighboring proteins (Figure 1B). Therefore, capturing early signaling (phosphorylation or dephosphorylation) immediately after cell stimuli could facilitate the detection of stimulus-specific phosphorylation.

To test this hypothesis, we chose the high-osmolarity glycerol (HOG) response pathway of the budding yeast *Saccharomyces cerevisiae* and performed rapid (5-s resolution) phosphoproteomics analyses to yield unprecedented profiling of HOG signaling (Chen and Thorner, 2007; Gustin et al., 1998; Saito and Posas, 2012). We chose to study this response because the signaling pathways are very well characterized and temporal responses have been studied in detail (Hersen et al., 2008; Muzzey et al., 2009; Saito and Posas, 2012). In addition, cell-to-cell variation of the response is very low so that dynamics of phosphorylation likely reflect responses of all cells in the whole population studied and not some subpopulation.

RESULTS

Dynamic Phosphoproteomics at Sub-minute Temporal Resolution

Common sample preparation protocols used in phosphoproteomics are not generally suited to the profiling of rapid signaling events because cell harvesting typically requires 5–15 min due to centrifugation and cell pellet washing steps. These steps can impart changes to the phosphoproteome that arise from both the stimulus of interest and the sample collection method. Thus, to assure that measured changes in the phosphoproteome are