IDENTIFYING THE HUMAN CALCINEURIN SIGNALING NETWORK

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Abstract

Systems-level analyses of phosphorylation-based signaling networks has transformed our understanding of kinase function, but knowledge of phosphatase signaling has lagged behind, primarily because global approaches to identify phosphatase substrates are lacking. Calcineurin, the conserved Ca^{2+}/calmodulin-dependent protein phosphatase and target of immunosuppressants, FK506 and Cyclosporin A, is ubiquitously expressed, and critically regulates Ca^{2+}-dependent processes in the immune system, heart, and brain. However, in the literature only ~30 human substrates are currently attributed to calcineurin.

We are using novel experimental and computational approaches to identify human proteins that contain “PxIxIT” and “LxVP” sequences. Calcineurin specifically recognizes its targets by binding to these short linear motifs (SLiMs), which occur preferentially in intrinsically disordered domains, and are challenging to identify due to sequence degeneracy and low affinity for calcineurin. Proteome peptide Phage Display (ProP-PD) was used to identify calcineurin-binding sequences of the PxIxIT and LxVP types experimentally from predicted disordered regions of the human proteome. These sequences directly identify novel and known calcineurin targets, and provide a large set of calcineurin-binding peptides for robust computational prediction of novel PxIxIT and LxVP-containing proteins in the human proteome. We also characterized the amino acid preference at each position of the PxIxIT and LxVP motifs using a novel technology that employs microfluidically-produced, spectraly-encoded beads, on which peptides are synthesized. Beads containing 96 distinct peptides, each with a unique spectral code, were incubated with calcineurin in a single volume and imaged to determine the amount of calcineurin that binds to each peptide.

Novel calcineurin-binding sequences that are identified either experimentally or computationally are validated using a high throughput calcineurin-binding assay, and their parent proteins tested for interaction with calcineurin in HEK-293 cells. These studies are identifying many new candidate

substrates for calcineurin that include ion channels, kinases, transcription factors and receptors, and reveal new points of cross-talk between calcineurin and other signaling pathways in human cells. Furthermore, these approaches can be broadly applied to systematic characterization of any SLiM-based signaling network.