Characterizing Suppressor of Cytokine Signaling (SOCS) SH2 domain binding specificity using phage immunoprecipitation sequencing (PhIP-Seq)

Timofey A. Karginov[1], Jonathan A. Cooper[2]

[1] The University of Chicago, Chicago, IL ; [2] Fred Hutchinson Cancer Research Center, Seattle, WA

BACKGROUND & SIGNIFICANCE

SOCS proteins are a well-studied group of substrate receptor subunits for the Cul5 E3 ubiquitin ligase, a protein critical for cell migration and signaling in epithelial cells. Preliminary data have tracked regulation of SOCS6 pathways by Src phosphorylation, a protein tyrosine kinase important for cell signaling. However, substrates phosphorylated by Src that bind to phosphotyrosine residues (pY) of the SH2 domain of SOCS6 and other SOCS proteins have not been established. Furthermore, SOCS SH2 domains have been poorly characterized due to their difficulty of synthesis in bacteria. Substrates of the SOCS-Cul5 RING ligase complex (CRL) have been identified as potential oncogenic proteins.

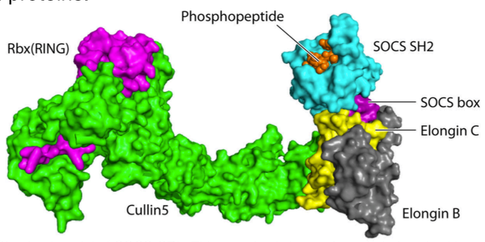


Figure 1: Structural model of SOCS-CRL. This complex is known to restrain tyrosine kinase signaling in epithelial cells and inhibit Src-dependent cell transformation. Image obtained from Ref 1. Cooper et al. Mol Cell Biol. 2015 Jun 1;35(11):1886-97.

In order to investigate specificity of SOCS binding proteins and identify consensus binding motifs to the SH2 domain after substrate phosphorylation by Src, we proposed to use PhIP-Seq method with a phage library containing 36 amino acid long open reading frame (ORF) sequences of the complete human genome.

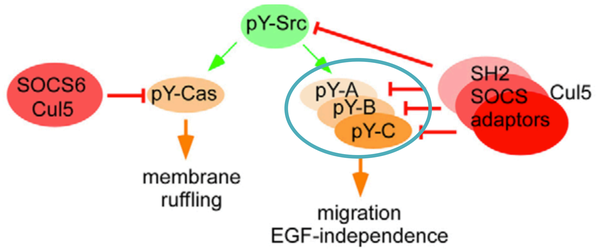


Figure 2: Model for the roles of Cul5, SOCS adaptors, and Src in epithelial cells. Unknown substrates of SOCS adaptors were found to be involved in cell migration. Image obtained from Ref 2. Teckchandani et al. J Cell Sci. 2014 Feb 1;127(Pt 3):509-20.

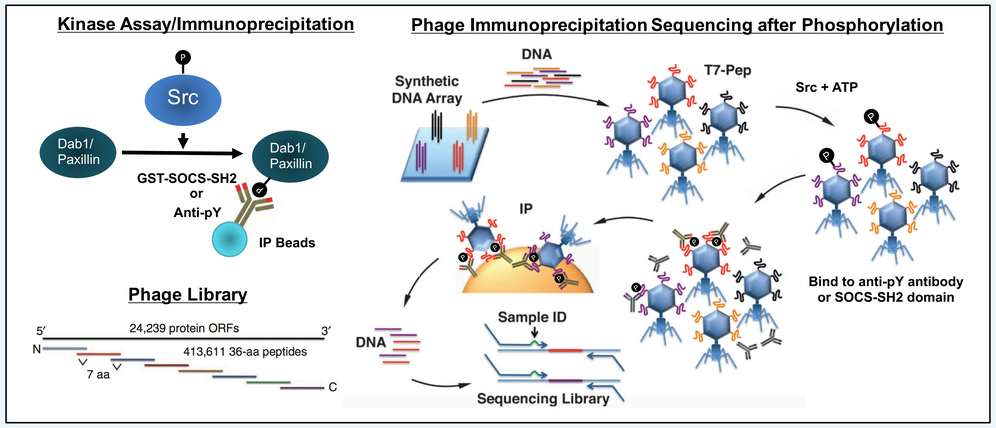
AIMS & APPROACH

1) **To investigate whether Src can function in the presence of the phage library.** Approach: Include known Src substrates, Dab1 and Paxillin, in Src kinase assays with and without phage.

2) **To measure stoichiometry Src substrate phosphorylation using immunoprecipitation in the presence and absence of the phage library.** Approach: Measure Paxillin bound to anti-pY 4G10 coated beads relative to total and unbound samples.

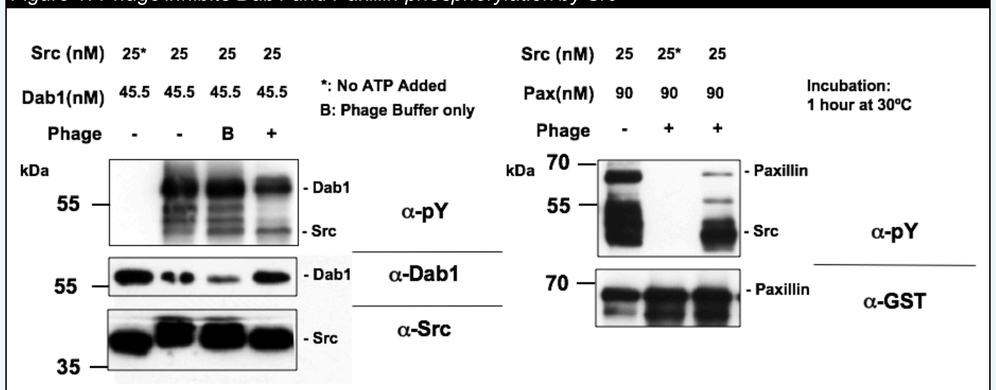
3) **To identify novel substrates of SOCS-SH2 domain using PhIP-Seq.** Approach: Develop GST-SOCS-SH2 domain constructs bound to Glutathione (GSH) beads to isolate phosphorylated phage for PhIP-Seq experiments.

CONCEPTS



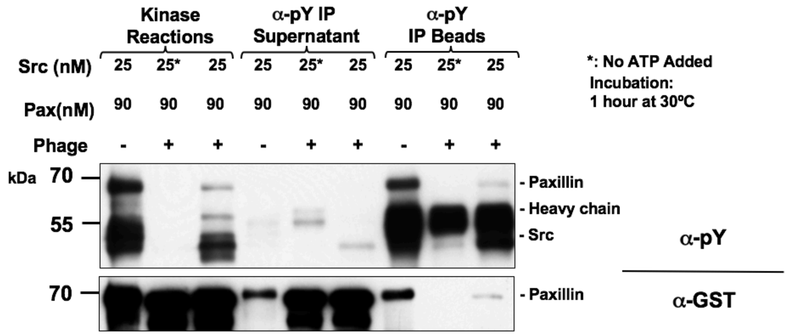
RESULTS

Figure 1. Phage inhibits Dab1 and Paxillin phosphorylation by Src



Src phosphorylates both Dab1 and Paxillin efficiently in absence of phage. In the presence of phage, substrate phosphorylation decreases while Src autophosphorylation is not significantly affected.

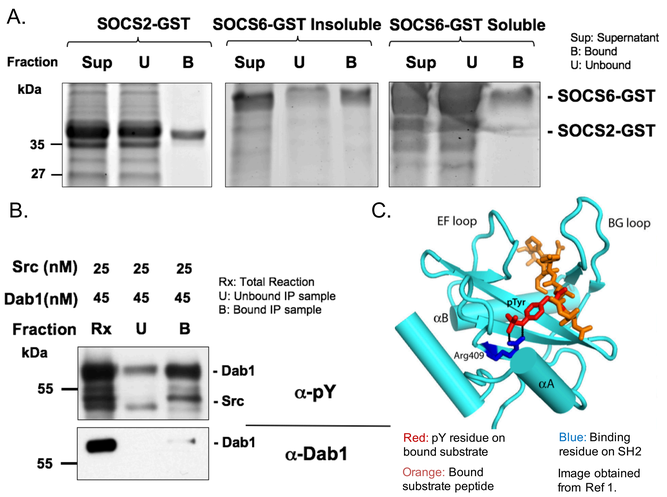
Figure 2. Immunoprecipitation of phospho-Paxillin shows phosphorylation stoichiometry



Anti-pY 4G10 precipitated a large fraction of phospho-Paxillin. Using anti-GST antibody to probe the Western, we estimate that approximately 20% of Paxillin is phosphorylated. Presence of phage did not inhibit precipitation of phospho-Paxillin but did inhibit phosphorylation.

RESULTS

Figure 3. SOCS SH2 Domain Constructs for PhIP-Seq



A. GST-SOCS SH2 proteins were expressed in bacterial cells. Cells were lysed and insoluble material was spun out. GST-SOCS2 SH2 appeared in the supernatant but a portion of GST-SOCS6 SH2 was in the pellet. Insoluble GST-SOCS6 SH2 was dissolved in urea and re-natured by dialysis. Both samples were bound to GSH-beads.

B. GST-SOCS6 SH2 beads were incubated with phosphorylated Dab1 substrate. A large fraction of pY Dab1 bound to beads, although this was only a small part of the total Dab1

C. Structure of SOCS6-SH2 bound to phosphorylated substrate.

CONCLUSIONS

- Src can phosphorylate substrates Dab1 and Paxillin, although its activity is inhibited in the presence of phage. Phage will be purified to address this issue.
- Immunoprecipitation experiments showed efficient binding of phospho-Paxillin to beads. Future PhIP-Seq experiments may require higher phosphorylation levels in the presence of phage.
- GST-SOCS6 SH2 binds effectively to phopho-Dab1, although phosphorylation levels of Dab1 are low compared to total Dab1. GST-SOCS6 SH2 will be used to bind phage library in order to then analyze new SOCS6 substrates using Next Generation Sequencing.

ACKNOWLEDGMENTS

Thank you to the Cooper lab for their support and efforts. This work is funded by RO1-GM109463. The Summer Undergraduate Research Program is supported in parts by the UCIHP Jeff Metcalf Summer Cancer Research Fellowship Program, the Cancer Center Support Grant (CCSG) CURE Supplement: 3 P30 CA015704-41S1, the Fred Hutch Internship Program, and individual labs/ research groups.