

**Stony Brook University
The Graduate School**

Doctoral Defense Announcement

Abstract

Determinants for Multisite Phosphorylation of p130Cas by Src

By

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p130Cas (Crk associated substrate, 130kDa) was first identified as a protein that is highly tyrosine phosphorylated in v-Src and v-Crk transformed cells. Cas is a multidomain protein with an N-terminal SH3 domain, a large central substrate domain containing 15 repeats of the YXXP motif, and a C-terminal Src binding region. The YXXP motifs can be subdivided into YDXP and YQXP motifs and they serve as substrates for protein tyrosine kinases such as Src. The Cas phosphorylation sites are important in cell migration, actin cytoskeleton organization, and Src-mediated anchorage independent growth. Previous studies from our laboratory showed that Src phosphorylates Cas by a processive mechanism and the major Src phosphorylation sites lie within the substrate domain of Cas. In these studies, we created mutant forms of Cas to identify the determinants for processive phosphorylation. We carried out a series of kinetic experiments using purified Src and Cas (wild-type and mutant forms). Our results show that mutants containing single or multiple YXXP mutations are phosphorylated processively by Src, suggesting that individual sites are dispensable. The results also suggest that there is no defined order to the Cas phosphorylation events. When we studied the effects of these mutations by re-introducing Cas into Cas-deficient fibroblasts, we observed that mutants lacking some or all YXXP sites can augment the ability of Src to promote anchorage independent growth. In contrast, deletion of the YXXP sites compromises the ability of Cas to promote tumor cell migration. In the second phase of the project, we wished to determine whether the arrangement and identity of phosphorylation motifs in the Cas substrate domain are important, or if (alternatively) any group of motifs arranged randomly can be phosphorylated by Src. To achieve this, we created a panel of Cas mutants in which the substrate domain is replaced by a random number and arrangement of the YXXP motifs. Our results indicate that these mutants are phosphorylated by Src *in vitro* and in intact cells, and can promote cell migration when introduced into Cas-deficient fibroblasts.

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