Novel Tyrosine Phosphorylation Sites Fine Tune the Activity and Substrate Binding of the Src Family Kinase Fyn

Marion E. Weir, Karen L. Hinkle, and Bryan A. Ballif

Department of Biology, University of Vermont, Burlington, VT, 05405, USA.

The protein Fyn is a member of the Src Family of Kinases (SFKs) that are important in many cellular processes including neuronal migration. Phosphorylation is an essential post-translational modification that has been previously shown to regulate kinase activity in SFKs. Many site-specific studies have identified tyrosine phosphorylation sites in SFKs that are both activating (e.g. Fyn Y420) as well as inhibitory (e.g. Fyn Y531) to SFK activity. These sites are located in the kinase domain and C-terminal regulatory domain of SFKs, respectively. More recently, we have utilized large scale mass spectrometry based proteomic approaches to identify novel phosphorylation sites in the SH2 (Src Homology 2) and kinase domain of Fyn that to date do not have known molecular functions. Using site-directed mutagenesis, we engineered constructs with phosphomimetic (Y to D) or non-phosphorylatable (Y to F) mutations for four of these sites, including Y185, Y213, Y214 (within the SH2 domain), and Y440 (within the kinase domain). Using in vitro and cellular approaches, we determined that SH2 domain phosphorylation at these sites increases Fyn's kinase activity while simultaneously reducing binding to other proteins. Conversely, we determined that phosphorylation of Y440 in the kinase domain reduces overall Fyn kinase activity. These results suggest that these previously uncharacterized phosphorylation events fine-tune SFK activity.