

ABSTRACT

Src kinase plays a crucial role in a multitude of fundamental cellular processes. Src is an essential component of signalling pathways controlling cellular proliferation, motility or differentiation, and is often found deregulated in tumours. Src activity is therefore maintained under stringent and complex regulation mediated by SH3 and SH2 domains and the phosphorylation state of tyrosines 416 and 527. Active Src adopts an open conformation whereas inactive state of the kinase is characterised by a compact structure stabilised by inhibitory intramolecular interactions. We identified phosphorylation of tyrosine 90 within binding surface of SH3 domain as a new regulatory switch controlling Src kinase activation. Using substitutions mimicking phosphorylation state of the residue we demonstrated that tyrosine 90 phosphorylation controls Src catalytic activity, conformation and interactions mediated by the SH3 domain, representing a positive regulatory mechanism leading to elevated activation of mitogenic pathways and increased invasive potential of cells. Based on correlation between compactness of Src structure and its catalytic activity, we constructed a FRET-based sensor of Src conformation enabling to measure the dynamics of Src activation in cells with spatio-temporal resolution. We found that activating mutations within either SH3, SH2 or kinase domains and some groups of inhibitors induce opening of Src structure. Analysing Src activity dynamics in focal adhesions we demonstrated that Src is activated during adhesion assembly, its activity remains steady and high throughout mature phase and decreases concurrently with adhesion disassembly.