JAK1 N-terminus binds to conserved Box1 and Box2 motifs of cytokine receptor common beta subunit but signal activation requires JAK1 C-terminus

Abstract

The human interleukin-3 receptor (hIL-3R) consists of a unique alpha subunit (hIL-3Ralpha) and a common beta subunit (betac). Binding of IL-3 to IL-3R activates Janus kinases JAK1 and JAK2. Our previously study showed that JAK2 and JAK1 were constitutively associated with the hIL-3Ralpha and betac subunits, respectively. In this study, we further demonstrate that JAK2 binds to the intracellular domain of hIL-3Ralpha and JAK1 binds to the Box 1 and Box 2 motifs of betac using GST-hIL-3R fusion proteins in pull-down assays. JAK1 mutational analysis revealed that its JH7-3 domains bound directly to the Box 1 and Box 2 motifs of betac. We further examined the role of JAK1 JH7-3 domains in JAK1 and JAK2-mediated signaling using the CDJAKs fusion proteins, which consisted of a CD16 extracellular domain, a CD7 transmembrane domain, and either JAK1 (CDJAK1), JAK2 (CDJAK2), or JAK1-JH7-3 domains (CDJAK1-JH7-3) as intracellular domains. Anti-CD16 antibody crosslinking of wild type fusion proteins CDJAK1 with CDJAK2 could mimic IL-3 signaling, however, the crosslinking of fusion proteins CDJAK1-JH7-3 with CDJAK2 failed to activate downstream proteins. These results suggest that the JAK1-JH7-3 domains are required for betac interaction and abolish wild type JAK1 and JAK2-mediated signaling.