

Molecular complexes that contain both c-Cbl and c-Src associate with Golgi membranes

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Abstract:

Cbl is an adaptor protein that is phosphorylated and recruited to several receptor and non-receptor tyrosine kinases upon their activation. After binding to the activated receptor, Cbl plays a key role as a kinase inhibitor and as an E3 ubiquitin ligase, thereby contributing to receptor down-regulation and internalization. In addition, Cbl translocates to intracellular vesicular compartments following receptor activation. We report here that Cbl also associates with Golgi membranes. Confocal immunofluorescence staining of Cbl in a variety of unstimulated cells, including CHO cells, revealed a prominent perinuclear colocalization of Cbl and a Golgi marker. Both the prominent Cbl staining and the Golgi marker were dispersed by brefeldin A. Subcellular fractionation of CHO cells demonstrated that about 10% of Cbl is stably associated with membranes, and that Golgi-enriched membrane fractions produced by isopycnic density centrifugation and free-flow electrophoresis are also enriched in Cbl, relative to other membrane fractions. The membrane-bound Cbl was hyperphosphorylated and it co-immunoprecipitated with endogenous Src. By immunofluorescence, some Src colocalized with Cbl and Golgi markers, and Src, like Cbl, was present in the Golgi-enriched fraction prepared by sequential density centrifugation and free-flow electrophoresis. Transfection of an activated form of Src, but not wild-type Src, increased the amount of Src that co-immunoprecipitated with Cbl, and increased the intensity of Cbl staining on the Golgi. This result, together with the increased tyrosine phosphorylation of the membrane-associated Cbl, suggests that Golgi-associated Cbl could be part of a molecular complex that contains activated Src. The localization and interaction of Src and Cbl at the Golgi and the regulation of the interaction of Cbl with Golgi membrane suggest that this complex may contribute to the regulation of Golgi function.

Keywords: c-Cbl; c-Src; Golgi apparatus