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**Structural and Functional Analysis of CIN85 and its Role in Receptor Endocytosis.**

**Abstract**

Endocytosis of receptor tyrosine kinases (RTKs) as epithelial growth factor receptor (EGFR) and c-Met/hepatocyte growth factor receptor is mediated by their ubiquitination by an intracellular enzyme ubiquitin ligase, Cbl. This is followed by the recruitment of other principal adaptor proteins such as **CIN85** and endophilins to form a **Cbl-CIN85-endophilin** complex which in turn regulates the endocytosis, lysosomal degradation and endosomal sorting of these RTKs.

Recent evidence shows that inhibition of the Cbl-CIN85-endophilin interaction was sufficient to block EGFR endocytosis and degradation leading to uncontrolled signalling and tumorigenesis. The present work focuses on the protein CIN85. It acts as a scaffold protein and is able to recruit signalling proteins into RTKs-associated complexes that are critical for RTKs endocytosis. Other than interacting with Cbl, CIN85 is found to non-covalently interact with a number of proline rich proteins/peptides as well as ubiquitin in the degradation pathway. The relatively low affinity of CIN85 for binding proteins could allow for rapid exchange of proteins/peptides, depending on their local concentration, cellular compartmentalization, or posttranslational modifications in response to changes in cell signal. Hence, our interest lies in ultimately determining the significance and contribution of these CIN85-binding protein interactions in the signalling cascade and its relation to tumorigenesis/cancer.

Different CIN85-C constructs have been cloned and expressed and its interaction with ubiquitin has presently been validated by GST-pull down assays, isothermal titration calorimetry and NMR experiments. Future work pertains to investigating the interaction of CIN85 with other interacting proteins/peptides and finally determine the structure of the complexes.