14-3-3 protein dependent modulation of ubiquitin ligase Nedd4-2

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Neural precursor cell expressed developmentally down-regulated 4 ligase (Nedd4-2) is an E3 ubiquitin ligase that targets proteins for ubiquitination and endocytosis, thereby regulating numerous ion channels, membrane receptors and tumor suppressors. In turn, Nedd4-2 activity is regulated by autoinhibition, calcium binding, oxidative stress, substrate binding (through its WW domains), phosphorylation and 14-3-3 protein binding [1-3]. However, the structural basis of 14-3-3-mediated Nedd4-2 regulation remains poorly understood.

Here, we combined several techniques of integrative structural biology to characterize Nedd4-2 and its complex with 14-3-3. The results from our binding affinity and crystallographic analyses demonstrate that phosphorylated Ser^{342} and Ser^{448} are the key residues that facilitate 14-3-3 protein binding to Nedd4-2 and that Ser^{448} is the dominant site. Moreover, 14-3-3 protein binding induces a structural rearrangement of Nedd4-2 by inhibiting interactions between its structured domains, including the N- and C-lobes of the catalytic HECT domain. Overall, our findings provide the first structural glimpse into the 14-3-3-mediated Nedd4-2 regulation and highlight the potential of the Nedd4-2:14-3-3 complex as a pharmacological target for Nedd4-2-associated diseases such as hypertension, epilepsy, kidney disease and cancer.

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