

Characterization of the dual-membrane specificity of rabphilin 3A

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Rabphilin 3A is a membrane traffic protein that contains a tandem C2AB-domain located in its C-terminal that is responsible for the Ca^{2+} -dependent phospholipid binding and mediates interactions with regulatory proteins like SNAP25, CASK, Annexin A4 and Miosin V. We report here functional analyses to characterize the molecular determinants of the Rabphilin3A interaction with membranes. By using Isothermal Titration Calorimetry we have determined the affinities and thermodynamic properties of these interactions and the results indicate that the C2AB domain binds preferentially to membranes containing PIP_2 and phosphatidylserine in the presence of Ca^{2+} . This is an exothermic reaction driven mainly by enthalpy changes. Dynamic Light Scattering assays have demonstrated that the main aggregation capacity resides in the C2B domain. Site-directed mutagenesis of key residues located at the different interacting surfaces of the C2AB domain shows that each one plays differential roles in the tandem. These is due to a collection of conserved key functional residues, but at the same time each one possess differential amino acids that confer them special abilities to interact with the membrane and with other proteins.

These findings provide functional explanation about how these domains are regulated by a dual-target mechanism and reveal how this family of proteins can employ subtle structural changes to modulate their sensitivity and specificity to various cellular signals.

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