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GABAC receptor rho subunits interact with PNUTS, a targeting subunit of protein phosphatase 1

Ralf Enz*[1], Heinrich Sticht[1] and Johann Helmut Brandstätter[1,2]

Address: [1]Institut für Biochemie, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany and [2]Institut für Biologie, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany

* Corresponding author

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Synaptic signal transduction depends on a precise regulation of neurotransmitter receptors by kinases and phosphatases. However, molecular mechanisms that specify the interaction between these proteins are largely unknown. Performing yeast two-hybrid screens using intracellular domains of GABAC receptor rho subunits as baits, we identified PNUTS, a nuclear targeting subunit of protein phosphatase 1 (PP1). PP1 contains several catalytic domains designated as PP1alpha, PP1beta/delta and PP1gamma1/2 that bind to a short motif in PNUTS composed of five amino acids. PNUTS interacted with one intracellular domain of the rho1/3 and with two intracellular regions of rho2. The rho interacting domains of PNUTS were different from its PP1 binding motif and consequently, PNUTS formed a ternary complex together with PP1gamma1 and rho2. Furthermore, we analyzed the structure of PNUTS in contact with the enzyme by homology-based molecular modeling. In the retina, immunohistochemical analys is showed expression of PNUTS in nuclei of cell bodies located in the inner nuclear layer and in the ganglion cell layer. In addition, Western blotting detected PNUTS outside the nucleus in cytosolic and membrane protein fractions of retinal cells. Specific label for the PNUTS binding partners PP1alpha, PP1beta, PP1gamma1 and GABAC receptors was mainly observed as clusters of immunofluorescent puncta in the synaptic layers of the retina. Finally, we show that PP1gamma1 and GABAC receptors are co-expressed in axon terminals of rod bipolar cells that express PNUTS. Based on our data, we suggest that PNUTS acts as a temporary bridge between GABAC receptors and catalytic domains of PP1.