NEURONAL CALCIUM SENSOR 1: UBIQUITOUS NEURONAL PROTEIN IN PHOTORECEPTOR SYSTEM

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Neuronal calcium sensor (NCS) 1 is a myristoylated membrane-binding protein capable of detecting Ca^{2+} signals and mediating various signaling pathways in neurons. NCS1 was commonly found in retinal neurons including rods and cones, but its function in photoreception remained unspecified. To discover unique features of photoreceptor NCS1, in this work we compared primary structure of NCS1 transcripts and protein products obtained from brain tissue and retina of two mammalian species, *Bos taurus* (bovine) and *Oryctolagus cuniculus* (rabbit). We also investigated localization of NCS1 in bovine photoreceptors, examined efficiency and mechanism of its interaction with photoreceptor membranes possessing unique phospholipid and protein composition, and identified potential protein targets of photoreceptor NCS1.

Examination of brain and retinal transcriptomes, using PCR-RACE technique, revealed previously unresolved complete mRNA sequences encoding bovine and rabbit NCS1. Although NCS1 transcripts from these species displayed single nucleotide polymorphisms, their translation would result in synthesis of polypeptides identical to full-length human NCS1. Meantime, mass-spectrometric analysis of immunoaffinity-purified bovine NCS1 revealed new peptides pointing to existence of N-terminal isoforms of the protein generated as a result of alternative splicing. Immunohistochemical analysis confirmed the presence of NCS1 in ganglion, inner and outer plexiform and photoreceptor layers of the retina and detected for the first time NCS1-specific staining in photoreceptor outer segments implying a role of this protein in phototransduction. Using membrane preparations, isolated from photoreceptor and brain (hippocampal) neurons, it was found that myristoylated NCS1 can bind to both membranes in a partially Ca^{2+}-independent manner. However, the interaction with photoreceptor membranes was more pronounced in the case of Ca^{2+}-free NCS1 indicating sensitivity of the protein to phospholipid composition of the membranes in the absence of calcium. This property of NCS1 was found to depend on the presence of positively charged lysine residues in N-terminus of the protein, foremost in the third position of its polypeptide chain. In addition, C-terminal segment of NCS1 was shown to be crucial for its Ca^{2+}-independent binding to photoreceptor membranes by underlying specific partially reversible Ca^{2+}-myristoyl switch of the protein. According to isothermal titration calorimetry studies, NCS1 can also interact with cytoplasmic domain of caveolin-1, integral protein of photoreceptor membrane rafts, and this interaction is significantly facilitated in the absence of Ca^{2+} ($K_D \approx 0.36$ μM). Using co-precipitation and mass-spectrometry analysis, three Ca^{2+}-dependent potential targets of NCS1 (GRK1, visual arrestin, tubulin beta 2A) as well as four Ca^{2+}-independent targets of the protein (V-ATPase D-subunit, S13 ribosomal protein, glyceraldehyde 3-phosphate dehydrogenase and recoverin) were identified in retinal extracts. Furthermore, *in vitro* studies indicated that NCS1 is capable of Ca^{2+}-dependent inhibition of phosphorylation of GRK1 substrates *in vitro*. Taken together, these data provide new insights into the role of NCS1 in photoreceptor system and extend current understanding of its general functionality in neurons.

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