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Novel GKAPs for cGMP-dependent protein kinase type II: identification and functional significance

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Many protein kinases are maintained in specific subcellular microenvironments by association to anchoring proteins. Proper functioning of the cGMP-dependent protein kinase II (cGK II) involves membrane targeting through myristoylation. To determine whether additional binding to anchoring proteins is also important for its targeting, in analogy with G-kinase anchoring proteins (GKAPs) for cGK I, and to identify potential new components of the cGMP/cGK signalling pathway we performed a yeast two-hybrid screen of novel cGK II binding partners. By screening a brain cDNA library using cGK II as a bait the intermediate filament protein GFAP (glial fibrillary acidic protein) was obtained as a positive clone. The interaction was confirmed by overlay- and pull-down assays and by immunoprecipitation. Interestingly, the highly homologous intermediate filament protein vimentin has previously been identified as a high affinity binding partner for cGK Iα. We determined which domains are required for the interaction and found, surprisingly, that the least conserved N-terminal domain of the cGK's (aa 1–108 of cGKII and 1–94 of cGK Iα) interacts with the most strongly conserved core domain of the intermediate filaments (aa 237–386 of GFAP). The physiological function of the interaction is under current investigation.

We have previously identified the PDZ domain protein NHERF-2 (E3KARP) as a low-affinity binding partner for cGK II and NHERF-2 binding was shown to be required for the cGMP-dependent inhibition of the sodium-proton exchanger NHE3 in the PS120 fibroblast cell line [1]. To determine whether NHERF-2 is also important for regulation of ion transport in native intestinal tissue we determined cGMP-dependent inhibition of fluid transport in NHERF-2 deficient mice. cGMP simultaneously activates chloride secretion through the cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel and inhibits salt uptake through NHE3. Our results show that whereas CFTR stimulation by 8-Br-cGMP was unaltered, the inhibition of salt- and fluid uptake in the small intestine was strongly reduced in NHERF-2 deficient mice suggesting that NHERF-2 functions as a GKAP for cGK II in native tissue.

References

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