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BIOGENESIS OF THE ATKCO3 POTASSIUM CHANNEL

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Plant cell homeostasis is maintained by the activity of channels and transporters. These proteins must be specifically targeted, sorted and retained at appropriate membrane domains to control the vectorial transport of fluid, solutes, and electrolytes. The extent of permanence at the site of action could also be regulated, through interactions with the cytoskeleton or other associated proteins. Therefore, targeting signals as well as signals that control turnover coexist on the polypeptide. We are studying the Arabidopsis AtKCO3 potassium channel as a model to identify targeting and turnover signals as well as possible interactors. AtKCO3 is a single pore channel with two transmembrane domains and the N- and C-terminal regions exposed in the cytosol. A 14-3-3 binding region and two EF-hands are predicted at the N- and C-terminal domains, respectively. An AtKCO3::GFP fusion was previously found to be located at the tonoplast by transient expression. By subcellular fractionation, we confirmed the tonoplast localization of overexpressed AtKCO3 and the AtKCO3::GFP fusion in Arabidopsis transgenic plants. We determined that both AtKCO3 and AtKCO3::GFP form dimers in transgenic plants and in transiently transfected protoplasts (from Arabidopsis colture or tobacco leaves). Because four pores are necessary in a functional channel, the results indicate that most probably KCO3 is not functional by itself. Our electrophysiological studies confirmed that KCO3 and KCO3::GFP are silent channels. To identify potential partners involved in the regulation of (or regulated by) KCO3 we are performing yeast two hybrid screening using the last 87 AA of KCO3 as bait. We also identified a putative PDZ-binding motif of class 1 (-X-S/T-X-F) at the C-terminus of AtKCO3. PDZ proteins act as adaptors that facilitate signaling or determine the localization of receptors, channels, transporters and other signalling molecules. We are determining the turnover and half-life of AtKCO3 and a mutated form deleted of the putative PDZ-binding domain.

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