The Role of Homer Family Adaptor Proteins in Regulation of Store-Operated Calcium Influx in A431 Cells

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The major pathways for Ca^{2+} influx in nonexcitable cells are store-operated channels (SOC). SOCs are activated by depletion of intracellular calcium stores. In the previous studies we used patch-clamp electrophysiology to describe the properties of store-operated channels $I_{\min}$ in human carcinoma A431 cell lines. $I_{\min}$ are gated by direct protein-protein interaction with inositol-1,4,5-trisphosphate receptor type 1 ($\text{IP}_3\text{R1}$). We supposed that this interaction might be facilitated by scaffold proteins of Homer family. Homer family proteins specifically bind to unique PPXXF sequence in IP_3R and in some other proteins. Several isoforms of Homer proteins exist. Homer 1c isoforms have coil-coiled domain, which mediate homooligomerization. That is why Homer 1c oligomers can form macromolecular complexes with partner proteins. Homer 1a isoform lacking coil-coiled domain can disrupt macromolecular complexes of Homer 1c and its partner proteins. The aim of this study was to determine the role of Homer protein in activation of store-operated channels.

We applied the Gst-Homer recombinant protein (concentration 100 nM) to inside-out patches excised

from A431 cells using 105 mM Ba^{2+} in the pipette as a current carrier. Application of Gst-Homer 1c to the cytosolic surface of the patch did not activate channels. Addition of GST Homer 1a to the same patch resulted in an activation of $I_{\min}$ channels.

In a similar series of inside-out recording experiments we used a synthetic peptide in place of Gst-Homer protein. We found that peptid PPKKFR activates $I_{\min}$, while control peptid PPKKRR has no effect.

By using single-channel patch-clamp recording we discovered that Homer proteins regulate the activity of low-conductance calcium selective store-operated channels $I_{\min}$ in plasma membrane of A431 cells. Moreover, oligomeric complexes of Homer 1c block the $I_{\min}$ activity, replacing complexes by Homer 1a isoform leads to activation of $I_{\min}$ channels without agonist stimulation by disruption of protein complex Homer 1c- IP_3R - $I_{\min}$. Interaction between Homer 1a and former partners of complex Homer1c- IP_3R - $I_{\min}$ is not critical for the $I_{\min}$ activation, the way of complex disruption is not important for $I_{\min}$ activity.