BIOCHEMISTRY 640

(Biomembranes Discussion Group)

Wednesday, March 15, 2017

Room 4-70 Medical Sciences Building

4:00 PM

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"Molecular Determinants in TRPV5 Channel Assembly"

The epithelial Ca^{2+} channels TRPV5 and TRPV6 mediate the Ca^{2+} influx in 1,25-dihydroxyvitamin D3-responsive epithelia and are therefore essential in the maintenance of the body Ca^{2+} balance. These Ca^{2+} channels assemble in (hetero)tetrameric channel complexes with different functional characteristics regarding Ca^{2+} -dependent inactivation, ion selectivity, and pharmacological block. Glutathione S-transferase pull-downs and co-immunoprecipitations demonstrated an essential role of the intracellular N- and C-tails in TRPV5 channel assembly by physical interactions between N-N tails, C-C tails, and N-C-tails. Patch clamp analysis in human embryonic kidney (HEK293) cells and $^{45}Ca^{2+}$ uptake experiments in Xenopus laevis oocytes co-expressing TRPV5 wild-type and truncated proteins indicated that TRPVΔ5N (deleted N-tail) and TRPVΔ5C (deleted C-tail) decreased channel activity of wild-type TRPV5 in a dominant-negative manner, whereas TRPV5ΔNC (deleted N-tail/C-tail) did not affect TRPV5 activity. Oocytes coexpressing wild-type TRPV5 and TRPV5ΔN or TRPV5ΔC showed virtually no wild-type TRPV5 expression on the plasma membrane, whereas co-expression of wild-type TRPV5 and TRPV5ΔNC displayed normal channel surface expression. This indicates that TRPV5 trafficking toward the plasma membrane was disturbed by assembly with TRPV5ΔN or TRPV5ΔC but not with TRPV5ΔNC. TRPV5 channel assembly signals were refined between amino acid positions 64–77 and 596–601 in the N-tail and C-tail, respectively. Pull-down assays and co-immunoprecipitations demonstrated that N- or C-tail mutants lacking these critical assembly domains were unable to interact with tails of TRPV5. In conclusion, two domains in the N-tail (residues 64–77) and C-tail (residues 596–601) of TRPV5 are important for channel subunit assembly, subsequent trafficking of the TRPV5 channel complex to the plasma membrane, and channel activity.