Phospholipase Cγ is essential for activation of store-operated Ca^{2+} channels in liver cells

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Release of Ca^{2+} from intracellular stores in non-excitable cells results in activation of Ca^{2+} influx through so-called store-operated Ca^{2+} channels (SOCs) on the plasma membrane (Putney *et al.*, 2001). Activation of these channels occurs in response to a decrease in the concentration of Ca^{2+} in the lumen of the endoplasmic reticulum, and it does not depend on how this decrease in $[Ca^{2+}]$ is initiated. The molecular mechanism that underlies this phenomenon is poorly defined. Phospholipase Cγ (PLCγ) has been previously shown to be either directly involved in activation of SOCs or to modulate their activity through the production of additional IP_3 in a number of cell lines (Patterson *et al.*, 2002). The identity of the SOCs regulated by PLCγ, however, has not been established.

In this work we used short interfering RNA (siRNA) to specifically reduce the expression of the genes encoding PLCγ1 and PLCγ2 and whole cell patch clamping technique to measure activation of store-operated Ca^{2+} current (I_{SOC}) in H4IIE liver cells. Immunofluorescence and Western blotting were employed to verify the effectiveness of siRNA and the time course of the knock down of PLCγ.

We have found that transfection of H4IIE liver cells with siRNA against PLCγ1 results in time dependent reduction of PLCγ1 protein with maximal effect apparent at 72-96 h. At the same time the amplitude of the I_{SOC} developed in response to intracellular perfusion with IP_3 in cells transfected with siRNA against either PLCγ 1 or 2 has decreased. The average maximal amplitude of I_{SOC} decreased from -3.3±0.2 pA/pF (n=23) in control cells to -2.3±0.3 pA/pF (n=15) in cells transfected with siRNA against PLCγ1 and to -1.5±0.25 pA/pF (n=13) in cells transfected with siRNA against PLCγ2. Co-transfection with two siRNAs against PLCγ1 and PLCγ2 together resulted in further reduction of the current to -0.65±0.17 pA/pF (n=14). Similar results were obtained when thapsigargin was used to activate I_{SOC} instead of IP_3. It is concluded that PLCγ is required for activation of I_{SOC} in liver cells, however, the catalytic activity of PLCγ in this process in not essential.

Putney, J.W., Jr., Broad, L.M., Braun, F.J., Lievremont, J.P. & Bird, G.S. (2001) *Journal of Cell Science* **114**, 2223-2229.

Patterson, R.L., van Rossum, D.B., Ford, D.L., Hurt, K.J., Bae, S.S., Suh, P.G., Kurosaki, T., Snyder, S.H. & Gill, D.L. (2002) *Cell* **111**, 529-541.