Metabolism of diacylglycerol on the cell membrane enhances cell signaling

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The ubiquitous second messenger Diacylglycerol (DAG) plays critical roles in multiple physiological and pathophysiological processes, such as lipid signaling, cell death, cytoskeletal dynamics, intracellular membrane trafficking, and neurotransmitter release. Currently, research work often focusses on the production and function of DAG on the plasma membrane; however, the detailed mechanism of DAG production and signaling at subcellular membranes is rarely investigated thoroughly. Our previous work reported that novel Protein Kinase C (nPKC) could target the ER membrane and consequently modulate Ca^{2+} mobilization [1]. Production of DAG on the ER membrane is probably responsible for such a targeting. To investigate this hypothesis, we applied confocal microscopy and genetically encoded sensors to monitor the metabolism of DAG in living cells. Interestingly we found that a PKC-C1-domain based DAG sensor accumulated on the ER membrane in naive HEK cells following activation of the cAMP-EPAC signal pathway. In contrast, following ATP stimulation we didn't observe any of DAG production at the plasma membrane. Although, P2Y-receptors activated by ATP triggered the $G_{\alpha}q$-PLCβ signaling pathway as indicated by intracellular Ca^{2+} release from the SR. Interestingly, following expression of a $G_{\alpha}q$ specific design receptor (DREADD) in HEK cells, the DAG sensor highlighted DAG production at the plasma membrane after activation of those receptors with their specific design drug (CNO). When expressing nPKCs we identified a similar translocation pattern for those PKC isoforms when compared to the DAG sensor. Furthermore, our experiment revealed that the lifetime of DAG on the plasma membrane appears to be much shorter than DAG on the ER membrane resulting in non-detectable DAG levels at the former membrane structure. These data strongly indicated that the dynamic metabolism of DAG on cellular membranes may contribute to differential targeting of C1 domain containing proteins and as such represents a novel mechanism of subcellular targeting of PKCs. These mechanisms thus substantially contribute to the cell´s signaling toolbox.

[1] Hui, X., Reither, G., Kaestner, L., Lipp, P., Mol. Cell. Biol. 34, 2370 (2014).