Role of lipid signalling pathways in an intra and an extracellular phenomenon

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Abstract

This dissertation is in two parts. The first part investigates the role of lipid signalling pathways in an intracellular phenomenon i.e. the formation of the nuclear envelope. The second part is a study of lipid signalling pathways in extracellular phenomenon, i.e. HeLa cell adhesion and spreading on a gelatin substrate. ^ The disassembly and formation of the nuclear envelope are crucial steps in the progression of mitosis. Nuclear envelope dynamics involve many steps and since vesicular transport, binding and fusion of vesicles are essential for the formation of the nuclear envelope it was our interest to explore whether there were any parallel pathways, such as found in the secretary pathways, that were also used in the formation of the nuclear envelope. There is little information on the proteins of membrane vesicles that reconstitute the nuclear envelope and practically none about their lipid composition. It was therefore important to determine their lipid structure in order to proceed with investigating whether, during the formation of the nuclear envelope, similar signalling pathways to those in the vesicle trafficking operate. ^ Cytoplasmic membrane vesicle fractions (MVs) from sea urchin eggs which, contribute to the nuclear envelope were studied. The phospholipid composition of the membrane vesicles, MV1, MV2 and MV3 was determined using a novel approach for direct quantification of phospholipids from two-dimensional ^{31}P-^{1}H nuclear magnetic resonance spectroscopy with isotropic mixing. MV2 and MV3 have similar compositions typical of the ER and the Golgi membranes. However, MV1 is mainly composed of phosphatidylinositol with phosphatidylcholine being the minor phospholipid present in MV1. ^ Furthermore, we determined that phosphatidylinositol specific phospholipase C (PI-PLC) promoted nuclear envelope formation. However, in the absence of MV1, PI-PLC, did not induce nuclear envelope formation. Inhibition of membrane vesicle fusion in a dose dependent manner, by wortmannin (a specific inhibitor of the PI-3kinase pathway) suggested that the PI-3 kinase branch of the phosphatidylinositol pathway may be involved in the formation of the nuclear envelope. These experiments indicate that the inositol phospholipid pathways, as in constitutive membrane trafficking, are also involved in the formation of the nuclear envelope. Furthermore, it is the first time that a biological membrane, MV1, with such an unusual composition has been reported and that it has been demonstrated the phosphatidylinositol hydrolysis is involved in the formation of the nuclear envelope. ^ In the second part the goal was to determine which lipoxygenase metabolites were involved in facilitating HeLa cell adhesion and spreading to a gelatin substrate. Reverse phase HPLC methods demonstrated that HeLa cell homogenates converted arachidonic acid into 12-, 15- and 5-hydroperoxyeicosatetraenoic (HETEs), indicating that 12, 15 and 5 lipoxygensases are active in HeLa cells. In order to investigate which lipoxygenase pathway are required to induce cell spreading the lipoxygenase pathway was inhibited by 25uM nordihydroguaiaretic acid (NDGA), a specific inhibitor of the lipoxygenases and

MK866, a specific inhibitor of leukotriene biosynthesis. ^ The effect of the different enantiomers of 12-BETE and 15-HETE on the reversal of NDGA inhibition was determined. The leukotrienes that overcome the inhibition of cell spreading by NDGA and MK866 were also determined. ^ It can be concluded that 12,15 and 5 lipoxygenase enzymes are present and active in HeLa cells; and 12-HETE, 15-HETE, leukotrienes LTB4, LTD4 and LTC4 play an active role in HeLa cell spreading on a gelatin substrate. ^

Subject Area

Molecular biology|Cellular biology|Biochemistry

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