tribution of individual metabolic pathways to the biosynthesis of natural products.

doi:10.1016/j.chemphyslip.2008.05.112

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Effects of inhibited PC synthesis on protein transport: The road less travelled

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The mutant Chinese hamster ovary cell line MT58 contains a thermo-sensitive mutation in CTP:phosphocholine cytidylyltransferase, the regulatory enzyme in the CDP-choline pathway. As a result, MT58 cells show a lower rate of phosphatidylcholine (PC) synthesis at the permissive temperature (32 °C) as compared to wild type cells, and an almost complete inhibition of PC synthesis at the non-permissive temperature of 40 °C. At the permissive temperature this lower rate of PC synthesis in MT58 cells is balanced by a lower rate of PC breakdown as compared to wild type cells. However, at the non-permissive temperature PC degradation is largely increased, resulting in a strong decrease in cellular PC. Despite this drastic decrease in cellular PC content, cells are still viable and can even proliferate if PC synthesis is restored by addition of lysoPC. By [^{3}H]-oleate labelling we found that the fatty acid moiety of the degraded PC is recovered in triacylglycerol, explaining the observed increase in this neutral lipid. In accordance with this finding, an accumulation of lipid droplets is seen in MT58 cells. Analysis of PC-depleted MT58 cells by electron and fluorescence microscopy revealed as well a dilation of the rough endoplasmic reticulum. Accompanied by this morphological observation our data demonstrate that inhibition of PC synthesis results in an impaired protein transport from the Golgi complex towards the plasma membrane.

doi:10.1016/j.chemphyslip.2008.05.113

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Selective visualization of fluorescent sterols in *Caenorhabditis elegans*

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Background: The nematode *Caenorhabditis elegans* (*C. elegans*) is an excellent genetically tractable model organism to investigate sterol physiology on the organism level. Fluorescence imaging of dehydroergosterol (DHE), a close cholesterol analog, has been recently introduced to characterize sterol transport in *C. elegans*.

Objectives: Detailed characterization of DHE distribution was hampered by *C. elegans*' high autofluorescence in the same spectral region as emission of DHE. We developed a new method to overcome this problem.

Methods: Using the rapid bleaching kinetics of DHE compared to cellular autofluorescence we were able to selectively detect DHE by wide field fluorescence bleach rate microscopy. Bleach kinetics of DHE were fitted on a pixel-basis to mathematical models describing the intensity decay. The developed method is compared with three-photon imaging of DHE in *C. elegans* and complemented with RNA interference experiments to knock-down target genes involved in sterol transport.

Results: Using this method we found enrichment of DHE in the intestine, neurons and the reproductive organs of *C. elegans*. We demonstrate compartmentalization of sterol in the oocytes and in intestinal cells and visualize sterol in gut granule loss (glo) mutant worms.

Conclusions: We developed a method to selectively visualize sterol distribution in vivo using fluorescence microscopy allowing for detailed investigation of sterol transport and metabolism on an organism level.

doi:10.1016/j.chemphyslip.2008.05.114