

Analysis of Novel Genes of the Mevalonate Pathway; Role of Peroxisomes in Cholesterol Metabolism

The isoprenoid biosynthetic pathway, is unrivaled in nature for the chemical diversity of the compounds it produces. Products from this pathway include all metabolically produced isoprene-containing compounds and sterols which provide chemical signals (hormones, pheromones) as well as structural components of enzymes (coenzyme Q and the isoprenoid moiety of heme *a*), and membranes (cholesterol, ergosterol). Mevalonate is also the precursor for farnesyl diphosphate (FPP) and geranyl-geranyl diphosphate (GGPP), both of which are required for the isoprenylation of various G proteins, most notable of which is the product of the *ras* gene, p21, a key transducer of mitogenic signals. The diversity of the isoprenoid products and their perturbation in cellular signaling, development and metabolic regulation suggests: 1) that regulation of isoprenoid biosynthesis has a pervasive influence on cell function and 2) there must be an intricate balance through which regulatory isoprenoid molecules are derived from a single biosynthetic pathway. It is to these two major proposals that our research is directed.

In mammalian cells, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase is the rate-limiting enzyme for the synthesis of mevalonic acid, the precursor of cholesterol and other non sterol isoprenoids. Because of its role in cholesterol biosynthesis, the regulation of HMG-CoA reductase has been intensely studied. The levels of the endoplasmic reticulum (ER) enzyme are governed by regulation of transcription, translation, and enzyme degradation. Another critical role for this enzyme has emerged in recent years, due to the requirement of FPP and GGPP in isoprenylation of proteins.

Analysis of a Novel HMG-CoA Reductase: We and others have demonstrated that HMG-CoA reductase is localized in two distinct intracellular compartments, ER and peroxisomes. No information is available regarding the function of the peroxisomal reductase in cholesterol/isoprenoid metabolism and the structure of the peroxisomal HMG-CoA reductase has yet to be determined. Accordingly, to facilitate our studies of the function and regulation of the peroxisomal HMG-CoA reductase and to determine its structure we have developed a mammalian cell line that expresses only one HMG-CoA reductase protein of 90 kDa and which is localized exclusively to peroxisomes. These cells provide a model system to study the peroxisomal HMG-CoA reductase independent of the ER reductase. The wild type CHO cells, contain two HMG-CoA reductase proteins, the well characterized 97 kDa protein, localized in the endoplasmic reticulum, and a 90 kDa protein localized in peroxisomes. Thus, our specific aims for this project are: 1) to study the regulation and function of the peroxisomal HMG-CoA reductase in this cell line; and 2) to isolate a cDNA encoding the peroxisomal HMG-CoA reductase.

Regulation of Cellular FPP Levels: FPP is a key intermediate that serves as a substrate for a number of critical branch-point enzymes, thus, the regulation and levels of FPP are important since large perturbations in FPP could alter the flux of isoprenoid compounds down the various branches of the pathway. We have recently made the significant finding that the entire pathway for the synthesis of FPP from mevalonate is localized in peroxisomes. This means that the cell's FPP is produced in the peroxisomes. Thus, FPP and/or farnesol has to be transported out of peroxisomes for further metabolism. In addition, phosphorylated products of mevalonate and isopentenyl are not able to cross the peroxisomal membrane. This implies that FPP is also impermeable and has to be transported out of the peroxisome. Therefore, we propose that regulated transport of FPP from its site of synthesis in peroxisomes into the cytoplasm plays a fundamental regulatory role in the utilization of FPP for sterol and non-sterol products. Thus, the major aims of this project are designed to answer important questions regarding the cellular levels, regulation and transport of FPP. The techniques utilized include protein biochemistry, cell biology and molecular genetics.

The Zellweger Mouse Model: The cerebro-hepato-renal syndrome of Zellweger is a fatal disease caused by deficient import of peroxisomal matrix proteins. The pathogenic mechanisms leading to death are unknown. Cholesterol levels and peroxisomal enzymes required for cholesterol biosynthesis are decreased in tissues from patients diagnosed with peroxisomal deficiency diseases. All of these diseases have severe neurological abnormalities whose pathophysiology is not known but may be related to cholesterol, since recent studies have shown that cholesterol is critical for normal brain development. However, very little information is available regarding cholesterol biosynthesis or compartmentalization of isoprene metabolism in the CNS.

We have recently demonstrated that in peroxisome assembly deficient Chinese Hamster Ovary cells (PEX2 mutants) the levels of cholesterol and the rates of cholesterol and dolichol biosynthesis are significantly reduced. Now, a peroxisomal PEX2 knockout mouse (Zellweger syndrome) has become available. These PEX2 deficient mice will provide an excellent model for studying the role of peroxisomal function in isoprenoid metabolism. Analysis of the PEX2 deficient animals may render insight into the mechanism(s) responsible for the neurological phenotype seen in the knockout mice.

We propose to: 1) Undertake a detailed analysis of the isoprenoid/cholesterol biosynthesis pathway in neonatal brain tissue; 2) Test for defects in lipid composition and cholesterol/dolichol metabolism in neurological tissues obtained from PEX2 deficient mice; and 3) Investigate if decreased cholesterol levels in PEX2 deficient mice impairs the sonic hedgehog signaling pathway.

Representative Publications

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