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Neuronal models for studying lipid metabolism and transport

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Abstract

New methods have been developed for studying lipid metabolism and transport in primary cultures of neurons. Sympathetic neurons from rats and mice, as well as retinal ganglion neurons from rats, can be cultured in three-compartmented culture dishes in which the cell bodies reside in a compartment separate from that housing the distal axons. In addition, the three compartments contain completely independent fluid environments. Consequently, these neuronal cultures represent an excellent model for studying the intraneuronal transport of lipids and proteins between cell bodies and distal axons. In addition, compartmented neuron cultures are particularly appropriate for investigating factors that regulate axonal growth and neuronal survival. The application of the compartmented culture model for use with murine neurons has opened up many new possibilities for studying lipid metabolism in neurons derived from genetically modified mice. Examples are given in which compartmented cultures of primary neurons have been used in studies on (i) lipid analysis of distal axons and cell bodies/proximal axons, (ii) immunoblotting of neuronal proteins involved in lipid metabolism, (iii) the compartmentalization of lipid metabolism, (iv) the role of lipids in axonal growth and survival, and (v) intracellular lipid trasport.
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1. Introduction

The study of lipid metabolism and transport in primary neurons poses some special challenges not encountered when investigating other types of cells such as cultured fibroblasts or hepatocytes, due to the highly elongated shape of the neurons. Nevertheless, these cells also offer distinct advantages for studying the compartmentalization of lipid metabolic processes and intracellular lipid trafficking. A neuron is a polarized cell that consists of distinct regions—the cell body, the axons, and the dendrites—each of which performs specific neuronal functions. The principal role of neurons is to transmit, conduct, and receive electrical signals. The axon terminals are the principal sites of neurotransmitter release whereas the dendrites are generally regarded as the sites at which the neurotransmitter signal is received. Thus, axons and dendrites contain a distinct distribution of proteins [1].

In a typical, growing sympathetic neuron in situ the growth cone of the axon moves forward at a rate of approximately 1 mm per day. It has been estimated that the plasma membrane of an axon expands at a rate of 1 μm²/min [2]. Thus, during axonal growth the supply of

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large amounts of membrane lipids and proteins is required for the expansion of axonal membranes. Secreted and membrane-bound proteins are transported from the cell bodies in lipid-containing vesicles along microtubules in the axons via fast axonal transport at a rate of approximately 200–400 mm per day. The transport of mitochondria into axons also occurs in this manner.

The membraneous organelles that make up the cell body include the plasma membrane, nucleus, mitochondria, endoplasmic reticulum, and Golgi apparatus [3], as well as numerous vesicles that are involved in the endocytic pathway, including lysosomes [4]. The synthesis of DNA and RNA occurs in the cell body, the location of the nucleus. Although the cell bodies are generally considered to contain all of the biosynthetic machinery required for axonal extension, axons have also been shown to contain mitochondria, elements of the endoplasmic reticulum [3,5], and the Golgi apparatus [6], as well as endocytic vesicles [7,8].

Most of the proteins in neurons are synthesized in the cell bodies and are transported via slow axonal transport (at a rate of 8 mm per day or less) into the axons [9,10]. However, compelling evidence has now demonstrated that some proteins (e.g., certain cytosolic metabolic enzymes and some abundant cytoskeletal proteins) can also be synthesized in axons [11–13] and dendrites [14,15]. Nevertheless, only a relatively few species of proteins are made in situ in the neurites. The production of these proteins in neurites is quantitatively much less than the fraction of these proteins made in cell bodies.

The most abundant membrane phospholipids (i.e., phosphatidylcholine, some of the phosphatidylethanolamine, phosphatidylserine, and cholesterol) in mammalian cells are synthesized on endoplasmic reticulum membranes [16]. In addition, a few classes of phospholipids (e.g., phosphatidylglycerol, cardiolipin, and some of the phosphatidylethanolamine) are synthesized in mitochondria. Sphingolipids, such as sphingomyelin and the complex glycosphingolipids, and the gangliosides, are particularly abundant in neurons and are, for the most part, made in the Golgi apparatus. Until relatively recently, the assumption had been that *all* membrane proteins and lipids required for the growth of axons and dendrites are synthesized in cell bodies and transported via anterograde transport into the neurites [17,18]. Although all indications are that cell bodies do, indeed, have the capacity to make all types of membrane lipids, it has now become apparent that not all of the lipids required for axonal growth need to be synthesized in the cell bodies and transported into the axons. For example, it is now known that at least 50% of the phosphatidylcholine in axons of rat sympathetic neurons is made in situ in the distal axons [19–21], whereas the remainder is presumably made in the cell bodies and exported to the growing axons. In addition, other membrane lipids, such as phosphatidylserine, phosphatidylinositol, and phosphatidylethanolamine (derived from both the CDP–ethanolamine pathway and from the decarboxylation of phosphatidylserine), as well as sphingomyelin, can be made in distal axons [19,20,22–24]. In contrast, cholesterol appears to be synthesized only in the cell bodies [20]. Consequently, an efficient transport process must exist for the transport of cholesterol to the membranes of distal axons for axonal growth.

This review will focus primarily on the methods that are currently available for studying lipid metabolism and transport in sympathetic neurons and retinal ganglion cells. Because of the polarized nature and highly elongated shape of neurons these cells provide excellent models for studying the compartmentalization of lipid metabolic processes and axonal lipid transport. We shall describe several neuronal model systems that can be used for studying intracellular lipid trafficking and metabolism and the relationship of these processes to neuronal survival and axon growth, focusing particularly on the use of primary neuron cultures isolated from rats and mice.

2. Models for culture of primary neurons

2.1. Neuronal cell lines

The use of commercially available neuronal cell lines has many advantages for performing standard biochemical and cell biological studies such as immunoblotting experiments, immunofluorescence localization studies, metabolic labeling studies, and enzymatic assays. These neuronal cell lines can be cultured under defined conditions and are readily amenable to transfection with cDNAs. In addition, gene "knock-down" experiments can be performed in these neuron-like cells with the use RNA interference techniques.

Two of the most commonly used neuron-like cell lines are PC12 cells and Neuro2a cells. PC12 cells are rat pheochromocytoma cells that are derived from a tumor of the adrenal medulla [25]. These cells have been well characterized and exhibit the interesting property that within 24 h of the addition of the neurotrophin, nerve growth factor, the PC12 cells cease dividing and adopt a neuronal phenotype that includes the sprouting and extension of neurites [25,26]. Thus, some properties of these cells resemble features of peripheral neurons. Neuro2a cells are murine neuroblastoma cells that can be induced to sprout neurites upon exposure to retinoic acid [27]. These cells, in some respects, resemble neurons of the central nervous system. In both PC12 cells and Neuro2a cells the rate of axonal extension can be assessed although the methodology is somewhat laborious and lacks precision. Nevertheless, despite the convenience of using these commercially available cell lines,

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