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Intracellular compartmentation of CTP synthase in *Drosophila*

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Abstract

Compartmentation is essential for the localization of biological processes within a eukaryotic cell. ATP synthase localizes to organelles such as mitochondria and chloroplasts. By contrast, little is known about the subcellular distribution of CTP synthase, the critical enzyme in the production of CTP, a high-energy molecule similar to ATP. Here I describe the identification of a novel intracellular structure containing CTP synthase, termed the cytoophidium, in *Drosophila* cells. I find that cytoophidia are present in all major cell types in the ovary and exist in a wide range of tissues such as brain, gut, trachea, testis, accessory gland, salivary gland and lymph gland. In addition, I find CTP synthase-containing cytoophidia in other fruit fly species. The observation of compartmentation of CTP synthase now permits a broad range of questions to be addressed concerning not only the structure and function of cytoophidia but also the organization and regulation of CTP synthesis.

Keywords: CTP synthase; cytoophidium; *Drosophila*; organelle; cilium; oogenesis

Introduction

A eukaryotic cell is divided into compartments such as the nucleus, the endoplasmic reticulum, the mitochondrion, Golgi bodies, and the cilium (Alberts et al., 2008). Compartmentation ensures increased structural and functional complexity within a cell's organization. Each compartment is functionally specialized, containing distinct catalytic processes, specialized molecules, and specific microenvironments. High concentrations of macromolecules localize to these compartments, allowing the cell to perform various metabolic activities efficiently and simultaneously. Specific products are transported between compartments using complex distribution systems (Alberts et al., 2008).

Nucleotides not only act as constituents of nucleic acids, but also serve as energy carriers, participate in cellular signalling, and act as important cofactors of enzymatic reactions (Nelson and Cox, 2000). Synthesis of nucleotides is tightly regulated within the cell. For example, ATP synthase, the enzyme responsible for ATP generation, is localized to mitochondria of animal cells and chloroplasts of plants and algae. The compartmentation of ATP synthase into organelles is very important for its function (Alberts et al., 2008).

CTP synthase is a glutamine amidotransferase enzyme that catalyzes the ATP-dependent transfer of the amide nitrogen from glutamine to the C-4 position of UTP to generate CTP (Lieberman, 1956; Long and Pardee, 1967). The CTP synthase reaction product CTP is an essential nucleotide and precursor for the synthesis of RNA, DNA, and sialoglycoproteins. CTP also plays an essential role in

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the synthesis of all membrane phospholipids in *Saccharomyces cerevisiae* and in mammalian cells. CTP is the immediate precursor of the activated, energy-rich phospholipid-pathway intermediates CDP-diacylglycerol, CDP-ethanolamine and CDP-choline. However, in contrast to previous extensive studies on the cellular organization of ATP synthase, little is known about whether and how CTP synthase is compartmentalized within the cell.

Here I describe the identification of a novel intracellular compartment containing CTP synthase in *Drosophila*. I detect CTP synthase using three antibodies specifically targeted to different regions of the CTP synthase molecule, as well as by two GFP-CTP synthase protein trap flies. I consistently detect filamentary structures in *Drosophila* cells using all five CTP synthase markers. I refer to these subcellular snake-like structures as cytoophidia (Greek: *cyto*-, meaning cell, and *ophidia*, meaning serpents). Multiple types of cytoophidia containing CTP synthase are detected in ovarian cells such as follicle cells, nurse cells and oocytes. In addition, I find that CTP synthase is compartmentalized within cytoophidia in cells from a wide range of *Drosophila melanogaster* tissues, such as brain, gut, testis, accessory gland, salivary gland, trachea, and lymph gland. Finally, I present evidence that cytoophidia containing CTP synthase are present in other species.

Materials and methods

Drosophila stock

Drosophila melanogaster stocks were raised at 21°C on standard cornmeal. The fly strain used in this study was *y w*. Protein traps of GFP-CTP synthase (CA06746 and CA07332) were provided by M. Buszczak and A. Spradling, Carnegie Institution of Washington, Baltimore, Maryland, USA (Buszczak et al., 2007). Fly stock GFP-SAS-4 was provided by J. Raff, University of Oxford, Oxford, UK (Dix and Raff, 2007).

Whole-mount tissue preparation

Tissues were dissected in Grace's medium (Invitrogen Ltd., Paisley, UK) and fixed in 4% paraformaldehyde in PBS at room temperature. Paraformaldehye was washed off with PBS for 30 min.

Immunostaining

Whole-mount tissues were stained with antibodies using a previously described protocol (Liu and Gall, 2007). The following antibodies were used in this study: Rabbit anti-CTP synthase (y-88, sc-134457), goat anti-CTP synthase (yD-18, sc-33304), and rabbit anti-phosphorylated CTP synthase (Ser36, sc-32829) were acquired from Santa Cruz Biotechnology Inc (Santa Cruz, CA, USA). One batch rabbit and two batches of rat anti-Cup sera were provided by A. Nakamura, Riken Center for Developmental Biology, Kobe, Hyogo, Japan (Nakamura et al., 2004). One batch from rat-anti Cup serum recognizes sperm tails and was renamed the "anti-Cup* antibody". Rat anti-Cup serum, mouse mAB anti-Lava Lamp (Actin-binding protein 2), and mAB anti-complex II alpha were provided by A. Spradling, Carnegie Institution, Baltimore, Maryland, USA (Keyes and Spradling, 1997). Mouse mAB anti-acetylated tubulin (T7451) was from Sigma-Aldrich Company Ltd., Dorset, UK. A mAB anti-gamma tubulin (T-6559) was also acquired from Sigma. Rabbit anti-SMN was provided by J. Zhou, University of Massachusetts, Amherst, Massachusetts, USA. Alexa 488-, Cy5- or Alexa-633 labelled goat or donkey anti-rabbit, rat or mouse IgG (Invitrogen) were used as secondary antibodies. DNA was stained with Hoechst 33342.

Confocal microscopy

Images were acquired under 40 × or 63 × objectives on a laser-scanning confocal microscope (Zeiss LSM 510 META, Oberkochen, Germany).

Results

Identification of novel filamentary structures, cytoophidia, in Drosophila

Both eIF4E and its binding partner Cup are involved in translation regulation (Nakamura et al., 2004). In *Drosophila* ovaries, the Cup protein colocalizes with eIF4E in the same structures, cytoplasmic processing bodies (P bodies) (Sheth and Parker, 2003; Wilhelm et al., 2003; Wilhelm et al., 2005; Liu and Gall, 2007; Lee et al., 2009). In an attempt to find P bodies in male gonads, I stained larval and adult testes using a number of P body