



ANNUAL REVIEWS **Further**

Click [here](#) to view this article's online features:

- Download figures as PPT slides
- Navigate linked references
- Download citations
- Explore related articles
- Search keywords

The Cytoophidium and Its Kind: Filamentation and Compartmentation of Metabolic Enzymes

Ji-Long Liu^{1,2}

¹Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford OX1 3PT, United Kingdom; email: jilong.liu@dpag.ox.ac.uk

²School of Life Science and Technology, ShanghaiTech University, Shanghai 201210, China; email: liujl3@shanghaitech.edu.cn

Annu. Rev. Cell Dev. Biol. 2016. 32:349–72

First published online as a Review in Advance on June 27, 2016

The *Annual Review of Cell and Developmental Biology* is online at cellbio.annualreviews.org

This article's doi:
10.1146/annurev-cellbio-111315-124907

Copyright © 2016 by Annual Reviews.
All rights reserved

Keywords

CTP synthase, CTPS, IMPDH, metabolism, organelle, cytoplasm, nucleus

Abstract

Compartmentation is essential for the localization of biological processes within a cell. In 2010, three groups independently reported that cytidine triphosphate synthase (CTPS), a metabolic enzyme for de novo synthesis of the nucleotide CTP, is compartmentalized in cytoophidia (Greek for “cellular snakes”) in bacteria, yeast, and fruit flies. Subsequent studies demonstrate that CTPS can also form filaments in human cells. Thus, the cytoophidium represents a new type of intracellular compartment that is strikingly conserved across prokaryotes and eukaryotes. Multiple lines of evidence have recently suggested that polymerization of metabolic enzymes such as CTPS and inosine monophosphate dehydrogenase into filamentous cytoophidia modulates enzymatic activity. With many more metabolic enzymes found to form the cytoophidium and its kind, compartmentation via filamentation may serve as a general mechanism for the regulation of metabolism.

Contents

INTRODUCTION.....	350
NOMENCLATURE.....	351
THE CYTOOPHIDIUM.....	351
CTPS.....	351
Evolutionary Conservation.....	352
Morphology.....	355
Composition.....	355
Occurrence and Dynamics.....	356
Assembly.....	356
Biogenesis.....	358
Physiological Functions.....	359
Links to Disease.....	361
THE IMPDH CYTOOPHIDIUM.....	361
IMPDH.....	361
Cytoophidia: IMPDH Versus CTPS.....	362
FILAMENTATION AND METABOLIC CONTROLS.....	364
Foci Versus Filaments.....	364
Compartmentation for Metabolic Regulation.....	365
The Benefit of Filamentation.....	366
CONCLUDING REMARKS.....	367

INTRODUCTION

“Compartmentation—the localization of catalysts, their substrates and products—is key to the transition from lifelike to living systems. This is evidenced by the cellular nature of all known life....”

Tolga Bilgen (2004)

Compartmentation is fundamental for a cell to function (Ovadi & Saks 2004, Sitte 1980). One method of compartmentation is to use membrane-bound organelles, such as the endoplasmic reticulum (ER), mitochondria, and the Golgi apparatus, which have been extensively studied for more than a century. Less well known is that macromolecules can be compartmentalized via the formation of membraneless structures (Brangwynne et al. 2009, Gall 2000, Hyman et al. 2014, O’Connell et al. 2012). For example, many non-membrane-bound organelles, such as cytoplasmic processing bodies (P bodies) (Sheth & Parker 2006), histone locus bodies (Liu et al. 2006a,b), uridine-rich small nuclear ribonucleoprotein bodies (U bodies) (Liu & Gall 2007), and purinosomes (An et al. 2008), have been identified inside the cell.

In the summer of 2010, three groups independently discovered that cytidine triphosphate synthase (CTPS), an essential metabolic enzyme responsible for the de novo synthesis of the nucleotide cytidine triphosphate (CTP), can form filamentous structures termed cytoophidia (Greek for “cellular snakes”) in *Drosophila* (Liu 2010), bacteria (Ingerson-Mahar et al. 2010), and budding yeast (Noree et al. 2010). The filament-forming property of CTPS is evidenced in human cells as well (Carcamo et al. 2011, Chen et al. 2011). The presence of CTPS-containing filamentous

structures across diverse species suggests that the formation of cytoophidia has an important biological function (Liu 2011).

The rate-limiting reaction for the de novo synthesis of another nucleotide, guanosine triphosphate (GTP), is catalyzed by inosine monophosphate dehydrogenase (IMPDH) (Hedstrom 2009). Interestingly, IMPDH forms filamentous structures (Carcamo et al. 2011) that appear very similar to the CTPS-containing cytoophidium. Moreover, a screening of GFP-tagged yeast strains showed that additional proteins can form filamentous structures (Noree et al. 2010). Extended screening in budding yeast recently identified many more metabolic enzymes with filament-forming capacity (Shen et al. 2016). Compartmentation via filamentation seems to be more general than we have appreciated in the past.

In this review, I begin by summarizing what we have learned about the cytoophidium that contains CTPS. Then, I discuss the IMPDH cytoophidium and its relationship with CTPS. Finally, I speculate on the benefit of the filamentation of metabolic enzymes.

NOMENCLATURE

The filamentous structures that contain CTPS have been termed cytoophidia, CTPS filaments, and cytoplasmic rods and rings (RR). For simplicity, the terms cytoophidium (singular) and cytoophidia (plural) are used in this review.

Cytoophidia are mesoscale, intracellular, filamentous structures that contain metabolic enzymes. If not specified otherwise, the term cytoophidium refers to the CTPS-containing filamentous structure, the first reported example of its kind. However, we can add a signature component to distinguish a specific subtype of cytoophidia. For example, the IMPDH cytoophidium is a filamentous structure that contains IMPDH, which may or may not contain CTPS.

A structure must meet several criteria to be referred to as a cytoophidium. First, it is a filamentous structure (in contrast to spherical bodies such as the P body, the U body, the Cajal body, and the histone locus body). Second, the structure generally contains metabolic enzymes (in contrast to classical cytoskeleton microtubules, microfilaments, and intermediate filaments). Third, the structure lacks a membrane (in contrast to membrane-bound organelles such as mitochondria, the ER, the Golgi apparatus, and cilia).

Depending on their relative size, cytoophidia can be subdivided into macrocytoophidia and microcytoophidia (Liu 2010). In *Drosophila* female germlines, macrocytoophidia are long and thick, whereas microcytoophidia are short and small. Microcytoophidia can undergo multiple rounds of fusion to form macrocytoophidia (Gou et al. 2014).

A eukaryotic cell might contain both cytoplasmic cytoophidia and nuclear cytoophidia (Carcamo et al. 2014, Gou et al. 2014, Shen et al. 2016, Zhang et al. 2014). If not specified, the term cytoophidia refers to cytoplasmic cytoophidia.

THE CYTOOPHIDIUM

CTPS

In many cells, CTP synthesis occurs through either the salvage pathway or the de novo pathway (Chakraborty & Hurlbert 1961, Kammen & Hurlbert 1959, Lieberman 1956, Long & Pardee 1967). CTPS catalyzes the rate-limiting step of de novo CTP biosynthesis (Chakraborty & Hurlbert 1961, Kammen & Hurlbert 1959, Lieberman 1956, Long & Pardee 1967). More specifically, CTPS catalyzes a set of three reactions: a kinase reaction [Mg^{2+} -ATP-dependent phosphorylation of the uridine triphosphate (UTP) uracil O₄ atom], a glutaminase reaction (rate-limiting

glutamine hydrolysis to generate ammonia), and a ligase reaction (displacement of the uracil O₄ phosphate by ammonia) (Endrizzi et al. 2004, 2005; Levitzki & Koshland 1971; Lewis & Villafranca 1989; von der Saal et al. 1985).

Early studies. In 1955, Liebermann first identified CTPS activity in *Escherichia coli* extracts that converted UTP to CTP and required ammonia, ATP, and Mg²⁺ (Lieberman 1955, 1956) (Table 1). Chakraborty & Hurlbert (1961) subsequently reported that glutamine is the primary amino donor of CTPS in *E. coli* (as it is in animal tissues) and that ammonia at higher concentration could be directly used by the enzyme in vitro. They also established that the requirement for a guanosine nucleotide is associated with the utilization of glutamine, but not with the utilization of ammonia. Long & Pardee (1967) purified CTPS approximately 300-fold and analyzed its kinetics with glutamine or ammonia as the nitrogen donor. In the same study, they also quantified the behavior of substrates and the allosteric activation by GTP (Long & Pardee 1967).

Between 1969 and 1972, Koshland and coworkers revealed a series of findings about CTPS (Genchev & Mandel 1976; Levitzki & Koshland 1969, 1970, 1971, 1972a,b; Levitzki et al. 1971; Long et al. 1970). They demonstrated negative cooperativity by effector GTP and substrate glutamine and analyzed tetramerization induced by glutamine and glutamine analogs. Although there are four binding sites for glutamine per tetramer, the affinity label 6-diazo-5-oxo-L-norleucine (DON, a glutamine analog) reacts with only half of the glutamine sites of CTPS, a finding that was subsequently explained by induced subunit interactions.

Subsequently, Scheit & Linke (1982) demonstrated that three main structural elements of the UTP molecule contribute to the substrate specificity of CTPS. Improved purification of CTPS by Anderson (1983) helped further elucidate its reversible cold lability and hysteresis properties.

Regulation. Four ribonucleotides directly interact with CTPS and regulate its activity (Robertson 1995). ATP is used to phosphorylate UTP, and both substrates bind CTPS with positive cooperativity (Anderson 1983, Endrizzi et al. 2004, Goto et al. 2004, Levitzki & Koshland 1972a, Long & Pardee 1967, Pappas et al. 1998, Robertson 1995). GTP binding allosterically activates glutamine hydrolysis and generates ammonia at a separate active site (Bearne et al. 2001, Endrizzi et al. 2004, Goto et al. 2004, Levitzki & Koshland 1972b, Scheit & Linke 1982). The uracil O₄ phosphate is displaced by ammonia to yield the CTP product, which provides negative feedback by competitively inhibiting the UTP substrate (Aronow & Ullman 1987; Endrizzi et al. 2004, 2005; Kizaki et al. 1985; Long & Pardee 1967; Yang et al. 1994).

Structure. In 2004, Baldwin and coworkers solved the crystal structure of *E. coli* CTPS at 2.3-Å resolution (Endrizzi et al. 2004). They found that each amidoligase active site and essential ATP- and UTP-binding surfaces are composed of three monomers, providing the structural evidence that CTPS activity requires oligomerization. A CTPS tetramer from another bacterium, *Thermus thermophilus*, adopts a similar cross-shaped structure, as revealed by Hirotsu and coworkers (Goto et al. 2004). Hirotsu and coworkers also proposed a model to explain the conformational change of the CTPS tetramer upon binding of ATP and UTP (Goto et al. 2004). Despite this extensive early work, the filament-forming property of CTPS was reported only within the past few years.

Evolutionary Conservation

In May 2010, CTPS was reported to be compartmentalized in filamentary structures termed cytoophidia in *Drosophila* (Liu 2010). Two months later, CTPS was described as forming filaments in *Caulobacter crescentus*, a bacterium exhibiting a curved shape (Ingerson-Mahar et al. 2010). A third

Table 1 A brief history of research on CTPS and cytoophidia

Year	Event	References
1955	CTPS activity found in <i>Escherichia coli</i> extracts; requirement of ammonia, ATP, and Mg^{2+}	Lieberman (1955, 1956)
1958–1959	Requirement of glutamine for CTPS activity in mammalian cells; requirement of GTP	Kammen & Hurlbert (1958, 1959)
1961	Requirement of glutamine or ammonia in <i>E. coli</i>	Chakraborty & Hurlbert (1961)
1967	CTPS purified 300-fold from <i>E. coli</i> extracts	Long & Pardee (1967)
1969–1972	Negative cooperativity; half-of-the-sites reactivity; kinetic effects of GTP	Levitzki & Koshland (1969, 1970, 1971, 1972a,b); Levitzki et al. (1971); Long et al. (1970)
1978	CTPS upregulated in cancer cells	Williams et al. (1978)
1982	Substrate specificity	Scheit & Linke (1982)
1983	Improved purification procedure	Anderson (1983)
1995	Determination of subunit dissociation constants	Robertson (1995)
1995–1996	Phosphorylation of CTPS by protein kinases A and C in yeast	Yang & Carman (1995, 1996), Yang et al. (1996)
2001	Targeting <i>Trypanosoma brucei</i> CTPS for the treatment of African sleeping sickness	Hofer et al. (2001)
2004–2005	Crystal structures of CTPSs from <i>E. coli</i> and <i>Thermus thermophilus</i> were solved	Endrizzi et al. (2004, 2005), Goto et al. (2004)
2008	Interacting proteins of human CTPS1 were identified	Higgins et al. (2008)
2010	Three groups (those of Ji-Long Liu, Zemer Gitai, and James Wilhelm) independently identified a novel intracellular CTPS structure termed the cytoophidium or CTPS filament in fruit fly, bacteria, budding yeast, and rat cells	Ingerson-Mahar et al. (2010), Liu (2010), Noree et al. (2010)
2011	CTPS-containing cytoophidia were identified in human cells; IMPDH-containing rods and rings (equivalent to cytoophidia) were identified in human cells	Carcamo et al. (2011), Chen et al. (2011)
2013	The N terminus of CTPS is necessary for filamentation	Azzam & Liu (2013)
2014	Discovery of cytoophidia in the nucleus	Carcamo et al. (2014), Gou et al. (2014), Zhang et al. (2014)
2014	Three reports show that filamentation downregulates CTPS enzymatic activity, although a fourth report suggests that filament formation upregulates CTPS enzymatic activity	Aughey et al. (2014), Barry et al. (2014), Noree et al. (2014), Strohlic et al. (2014)
2014	Human CTPS1, not CTPS2, plays a critical role in B lymphocyte proliferation	Martin et al. (2014)
2014	Identification of nuclear cytoophidia and cytoplasmic cytoophidia in <i>Schizosaccharomyces pombe</i> ; asymmetric inheritance of cytoophidia in <i>S. pombe</i>	Zhang et al. (2014)
2015	CTPS and IMPDH form independent filamentous structures	Keppeke et al. (2015)
2015	CTPS plays a critical role in brain development	Tastan & Liu (2015)
2015	Filamentation of IMPDH upregulates its activity	Chang et al. (2015)
2015	The proto-oncogene Cbl regulates cytoophidium formation in <i>Drosophila</i>	Wang et al. (2015)
2016	The oncogene Myc regulates cytoophidium formation in <i>Drosophila</i>	Aughey et al. (2016)
2016	Identification of 23 filament-forming proteins in a screening of 4,159 proteins in <i>Saccharomyces cerevisiae</i>	Shen et al. (2016)

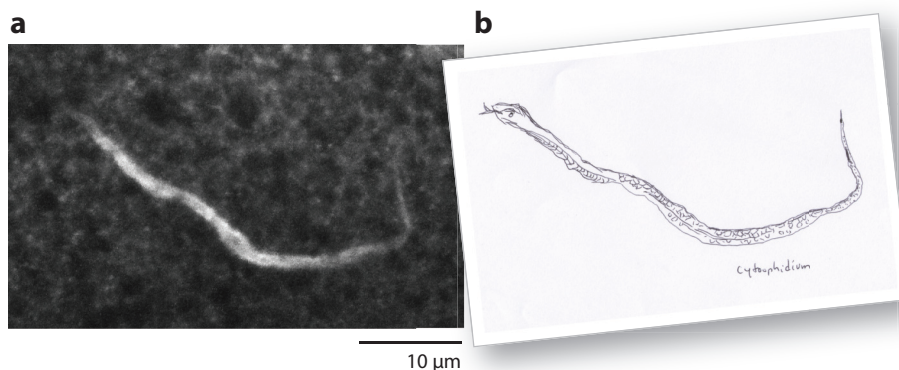


Figure 1

The cytophidium: a snake in the cell. (a) A snake-like structure observed in a *Drosophila* oocyte. This was one of the first images of cytophidia obtained by antibody cross-reaction. Adapted with permission from Liu (2010). (b) A drawing of a snake mimicking the image in panel a.

paper published in August 2010 suggested that CTPS proteins in the budding yeast *Saccharomyces cerevisiae* also form filaments (Noree et al. 2010). Given that the biochemistry and structure of CTPSs have been intensively studied over the past six decades, it came as a surprise that CTPS molecules form such an unusual feature.

Fruit flies. My observations of the cytophidium in *Drosophila* started from a serendipitous antibody cross-reaction several years ago (Liu 2010). Initial efforts focused on a translation initiation complex protein, Cup, which I used as a marker for the P body (Lee et al. 2009, Liu & Gall 2007). Fruit fly tissues were stained using multiple anti-Cup antibodies from different sources, and one was found that showed enigmatic filamentary structures in nurse cells, oocytes, and follicle cells. I referred to these novel structures as cytophidia owing to their serpentine forms (**Figure 1**). The cytophidium appears to be the same structure revealed by two protein trap lines in which CTPS was tagged by GFP, and by three antibodies specifically against different regions of the CTPS protein (Buszczak et al. 2007, Liu 2010). Moreover, I found that CTPS-containing cytophidia are present in many tissues, including the brain, gut, trachea, testis, accessory gland, salivary gland, and lymph gland (Liu 2010). Additionally, cytophidia were observed in other fruit fly species (Liu 2010).

Bacteria. Gitai and coworkers were interested in the cytoskeleton of *C. crescentus*, a bacterium exhibiting a unique curved morphology and asymmetric division cycle (Ingerson-Mahar et al. 2010). A bundle of filaments along the inner curvature had previously been identified by electron cryotomography (Li & Jensen 2009). To identify the filament-forming proteins, these investigators searched a collection of tagged proteins for nondiffuse localization and found that CTPS exhibited filamentary structures along the inner curvature of the cell (Ingerson-Mahar et al. 2010). By manipulating the activity of CTPS, they showed that CTPS regulates cell shape. Moreover, they demonstrated that CTPS protein from *E. coli* also forms filaments in vivo and in vitro (Ingerson-Mahar et al. 2010).

Budding yeast. A previous partial screen of a yeast GFP collection identified 33 proteins showing large punctuate structures (Narayanaswamy et al. 2009). Using a similar strategy, Wilhelm and

colleagues partially screened the GFP collection and found four types of filaments, including the *S. cerevisiae* CTPS filament (Noree et al. 2010). They identified some environmental conditions that regulate filament formation in budding yeast. They also demonstrated that CTPS localizes to filamentary structures in *Drosophila melanogaster* and in rat hippocampal neurons (Noree et al. 2010).

Subsequent studies have shown that CTPS can also form filaments in human cells (Carcamo et al. 2011, Chen et al. 2011) and fission yeast (Zhang et al. 2014). Thus, the cytoophidium represents a new type of intracellular compartment that is strikingly conserved across prokaryotes and eukaryotes (Liu 2011).

Morphology

In an attempt to identify novel filaments, Gitai and coworkers discovered that CTPS can form filaments (Ingerson-Mahar et al. 2010). *C. crescentus* has two types of cells: the stalked cell and the swarmer cell. The cytoophidium is nucleated at the central region of newly formed stalked cells. During stalked cell growth, the filament elongates to 500 nm long (whereas the cell length is approximately 1,000 nm). The filaments later move toward the periphery of the cell, i.e., the inner cell curvature.

In budding yeast, both CTPS proteins, Ura7p and Ura8p, can form foci and filaments (Noree et al. 2010). As the diameter of budding yeast cells is 4–7 μm , the average length of CTPS cytoophidia is 2–3 μm . These cytoophidia appear to be straight and stubby.

Cytoophidia can be observed in all three major cell types in *Drosophila* ovaries (Azzam & Liu 2013, Liu 2010, Noree et al. 2010, Strohlic et al. 2014, Wang et al. 2015). In early- and middle-stage egg chambers, each follicle cell contains one predominant cytoophidium. In germline cells, there are two types of cytoophidia detectable under light microscopy (Liu 2010). The large and thick macrocytoophidia can reach 30–40 μm long, whereas hundreds of thousands of microcytoophidia in a germline cell are small and short at 1–3 μm in length. Macrocytoophidia are made of a number of thin filaments. Multiple bundles with gaps in between can be observed in some macrocytoophidia. Along the long axis of macrocytoophidia, CTPS can be discontinuous with some gaps, suggesting that additional components exist in cytoophidia. Microcytoophidia morphologically connect with the Golgi apparatus, although whether cytoophidia and the Golgi apparatus are functionally coupled remains unclear (Liu 2010).

Most cytoophidia in *Drosophila* germline cells are linear. In contrast, cytoophidia in larval lymph glands are frequently shown as ring shaped or C shaped. It is still unknown how circular cytoophidia differ functionally from linear ones (Liu 2010).

CTPS can form cytoophidia in human cells in both the cytoplasm and the nucleus (Gou et al. 2014). Both cytoplasmic and nuclear cytoophidia can be observed in the fission yeast *Schizosaccharomyces pombe* (Zhang et al. 2014).

Composition

The first known component of the cytoophidium is CTPS (Ingerson-Mahar et al. 2010, Liu 2010, Noree et al. 2010). To reveal the composition of cytoophidia, one classical approach is subcellular fractionation. Large cytoophidia can form in culture cells when CTPS is overexpressed (Aughey et al. 2014, Gou et al. 2014). These large cytoophidia can be biochemically purified. Another possible approach is genome-wide screening of fluorescence-tagged proteins to search for filament-forming proteins (Narayanaswamy et al. 2009, Noree et al. 2010, Shen et al. 2016).

Ting's lab introduced engineered ascorbate peroxidase (APEX) as a genetic tag that harnesses the power of microscopy and mass spectrometry (Lam et al. 2015, Martell et al. 2012, Rhee et al.

2013). This new technology enabled this group to perform proteomic mapping of intracellular compartments that might not be feasible through classical approaches. For example, the group was able to identify proteins residing in the mitochondrial interspace, an area that is impossible to purify by subcellular fractionation (Hung et al. 2014). The APEX technology can be used to map the proteome of cytoophidia.

Occurrence and Dynamics

In the curved bacterium *C. crescentus*, CTPS prefers to form filamentous structures in the stalked cells rather than in the swarmer cells (Ingerson-Mahar et al. 2010). Thus, the frequency of cytoophidia can be variable in different cell types. During cell division in *S. pombe*, only one of the two daughter cells inherits the cytoophidium from the mother cell, whereas the other daughter cell synthesizes a new cytoophidium (Zhang et al. 2014). In multicellular organisms, the occurrence of cytoophidia seems complicated. In *Drosophila* ovaries, cytoophidia exist in all three major cell types, i.e., nurse cells, oocytes, and follicle cells (Liu 2010). However, the occurrence of cytoophidia is presented differentially along the developmental stages. In germline cells, both macro- and microcytoophidia appear at the early stages and continue to be present until middle oogenesis. Macrocytoophidia decrease at stage 10B and then disappear in the later stages (Liu 2010). However, microcytoophidia can be detected in stage 14 egg chambers (Chen et al. 2011). In follicle cells, cytoophidia emerge from the early stages to stage 10A but disassemble at stage 10B. No cytoophidia are detectable in stage 11–14 follicle cells, correlating with the expression of the transcription factor Myc (Aughey et al. 2016). In the larval central nervous system, cytoophidia occur in early-stage neuroblasts (Aughey et al. 2014). Cytoophidia disassemble upon neuroblast reactivation. In the third-instar larval stage, cytoophidia are present in the neuroepithelium (Chen et al. 2011, Tastan & Liu 2015).

Mouse embryonic stem cells contain abundant ring-shaped cytoophidia (Carcamo et al. 2011). These cytoophidia disassemble upon differentiation. This finding indicates that cytoophidia preferentially arise in fast-growing cells. The Wilhelm lab has observed that cytoophidia preferentially occur in the axons, but not in the dendrites, of rat neurons (Noree et al. 2010).

Cytoophidia are motile. In *C. crescentus*, CTPS filaments translocate from the cell center to the periphery and are eventually anchored in the inner curvature (Ingerson-Mahar et al. 2010). In *S. pombe*, cytoplasmic cytoophidia move constrainedly in certain regions, whereas nuclear cytoophidia act at the periphery of the nucleus (Zhang et al. 2014). The dynamics of cytoophidia have also been studied in budding yeast and mammalian cells (Gou et al. 2014, Shen et al. 2016).

Assembly

To determine the function of the cytoophidium, it is necessary to understand the assembly process and its regulation in detail. Although the assembly process can be studied via pharmacological approaches combined with live imaging, *Drosophila* oogenesis provides an excellent model for the regulation of cytoophidium assembly. *Drosophila* ovaries have been extensively studied in genetics and in cellular and developmental biology.

Assembly phases. The assembly of cytoophidia can be divided into five phases: nucleation, elongation, fusion, bundling, and circularization (**Figure 2**). The assembly of cytoophidia can be monitored by fluorescence microscopy. Live imaging of mouse NIH/3T3 cells expressing GFP-fused CTPS has revealed multiple phases of cytoophidium assembly (Gou et al. 2014). Previous studies have shown that DON promotes cytoophidium assembly (Carcamo et al. 2011, Chen et al. 2011).

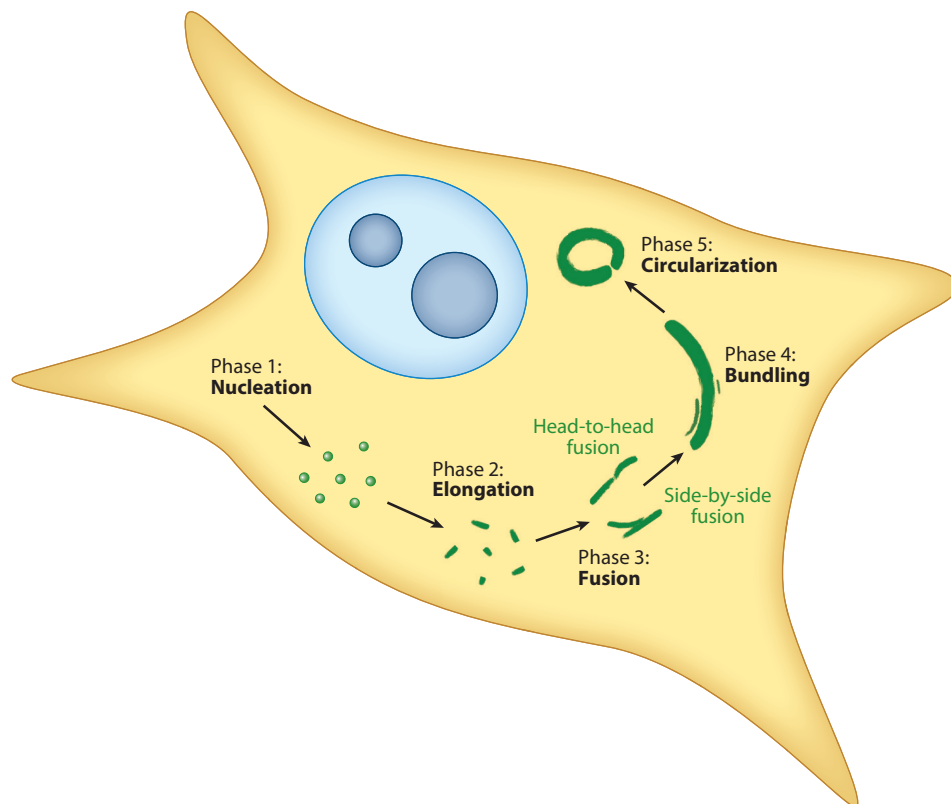


Figure 2

The five phases of cytoophidium assembly: (1) nucleation, (2) elongation, (3) fusion, (4) bundling, and (5) circularization. Modified from Gou et al. (2014).

After treatment with DON, the first nucleation phase is characterized by multiple foci forming simultaneously in the cytoplasm (Gou et al. 2014). The foci initially have spherical structures but elongate in the second phase. When their long axes reach several micrometers, small cytoophidia are dynamic and process to the third fusion phase (Gou et al. 2014). There are two major types of fusion. One type is head-to-head fusion, which increases the overall length while maintaining a similar thickness. The other type is side-by-side fusion, which increases cytoophidium thickness. The orientations of cytoophidia are frequently changed. For side-by-side fusion, cytoophidia seem to slide toward each other. This sliding movement suggests that cytoophidia can be driven along cytoskeletal tracks. Fusion can happen for multiple rounds. Middle-sized cytoophidia undergo the fourth phase (bundling). The cytoophidia become very long and thick. Long and thick linear cytoophidia can sometimes go through the fifth phase (circularization), in which both ends of linear cytoophidia fuse together.

CTPS level. CTPS level is critical for cytoophidium assembly. When RNAi is used to knock down CTPS, cytoophidia in follicle cells disassemble (Chen et al. 2011). In contrast, overexpressing CTPS promotes cytoophidium assembly (Azzam & Liu 2013). In follicle cells, overexpressed CTPS increases the length and thickness of cytoophidia. The cytoophidia become C shaped or O shaped because they are constrained inside the cell. In germline cells, overexpressed CTPS

induces extraordinarily long cytoophidia, many of which are bundled and tangled together. The abundance of CTPS also affects filament assembly in *C. crescentus* (Ingerson-Mahar et al. 2010). In the wild-type situation, CTPS filaments localize at the inner curvature of this curved bacteria. Mild overexpression increases the length of CTPS filaments, whereas strong overexpression can not only increase the length but also thicken the diameter (Ingerson-Mahar et al. 2010). These data suggest that increasing CTPS levels affects first the elongation phase, and then the fusion and bundling phases.

Myc. In *Drosophila* follicle cells, cytoophidia occur from the early stage until stage 10A, which is consistent with the expression level of the proto-oncogene Myc (Aughey et al. 2016). Cytoophidia disassemble in follicle cells at stage 10B, when Myc expression dramatically drops. Cytoophidia remain undetectable in follicle cells during late oogenesis (i.e., from stage 11 to stage 14), when little Myc is expressed. Myc appears necessary for cytoophidium assembly. In early- and middle-stage follicle cells, knocking down Myc results in cytoophidium disassembly. Conversely, overexpressing Myc leads to increased cytoophidium length during early and middle oogenesis. In late-stage follicle cells, overexpressing Myc can induce de novo assembly of cytoophidia. The occurrence of cytoophidia also correlates well with Myc expression in other tissues such as brains and imaginal discs in *Drosophila* larvae. In human cells, Myc-binding sites have been identified at the CTPS gene locus, suggesting a potentially direct role of Myc in CTPS transcription (Liu et al. 2008, Wu et al. 2008). Consistent with this idea, overexpression of Myc increases CTPS mRNA levels in *Drosophila*, as revealed by quantitative PCR (qPCR) (Aughey et al. 2016), and CTPS mRNA levels decrease when Myc is knocked down.

Cbl. In the search for additional regulators of cytoophidium assembly, Pai and coworkers treated *Drosophila* ovaries with MG132, a proteasome inhibitor (Wang et al. 2015). The rationale was that the inhibition of proteasome-mediated degradation of CTPS would promote cytoophidium assembly. Surprisingly, they observed that inhibition of the proteasome caused cytoophidium disassembly in follicle cells and germline cells, suggesting that ubiquitination plays a positive role in cytoophidium assembly. Consistent with this observation, treatment with the deubiquitinase inhibitor Pr619 helps to preserve cytoophidium assembly against the MG132 treatment. Furthermore, they showed that Cbl, an E3 ubiquitin ligase in follicle cells, plays a critical role in regulating cytoophidium assembly.

Ack. Peterson and colleagues showed that *Drosophila* Ack kinase (DAck), the ortholog of non-receptor tyrosine kinase Ack, colocalizes with CTPS in female germline cells, although DAck seems absent in follicle cells (Strochlic et al. 2014). They showed that the nurse cell membrane is disrupted in DAck mutants and that the membrane defects are linked to reduced CTPS activity. DAck mutation does not totally inhibit cytoophidium assembly. In nurse cells, cytoophidia become fragmented with short length, whereas the number of cytoophidia increases. These data indicate that DAck acts as a glue for the integrity of macrocytoophidia in *Drosophila* germline cells.

Biogenesis

How is the cytoophidium formed? Perhaps *S. pombe* can give us a clue. In each *S. pombe* cell, there is one cytoplasmic cytoophidium and one nuclear cytoophidium (Zhang et al. 2014). During cell division, the single cytoplasmic cytoophidium from the mother cell is inherited by one of the two daughter cells. Similarly, the single nuclear cytoophidium from the mother cell goes to one of the two nuclei in the daughter cells. The asymmetric inheritance of both cytoplasmic and

nuclear cytoophidia appears to be a stochastic process. In addition, the inheritance of the nuclear cytoophidium is independent of that of the cytoplasmic cytoophidium. Cytoophidia form de novo in the daughter cell that does not inherit the cytoophidium from the mother cell.

In *Drosophila* female germline cells, microcytoophidia are linked to the Golgi apparatus (Liu 2010). It is not known whether the Golgi apparatus and cytoophidia are functionally connected. The origin of cytoophidia may be tracked to the Golgi apparatus and the ER. One possible scenario for the biogenesis of cytoophidia can be depicted as follows. After being synthesized at the ER, new CTPS proteins are potentially modified at the Golgi apparatus and nucleate into small foci. Then these small foci elongate and fuse into a large one.

Physiological Functions

Compartmentation within organelles has been recognized for many years as a major way by which a cell can efficiently carry out various processes. The discovery of the cytoophidium across prokaryotes and eukaryotes is potentially fundamental and important. An integrated understanding of the biology of the cytoophidium and its kind will deepen our understanding of the cell biology of metabolism. I would like to speculate on the physiological functions of cytoophidia as follows (Figure 3).

1. **Cytoskeleton-like function.** In *C. crescentus*, CTPS filaments cooperate with the intermediate filament CreS to maintain cell shape (Ingerson-Mahar et al. 2010). This role, however, must be secondary, as filamentous CTPS occurs in rod-shaped *E. coli* (Ingerson-Mahar et al. 2010) and in spherically shaped cells such as budding yeast (Noree et al. 2010).
2. **Metabolic control.** Several studies indicate that forming cytoophidia is a way to regulate metabolism (Aughey et al. 2014, Barry et al. 2014, Noree et al. 2014, Strohlic et al. 2014). Filamentation of metabolic enzymes can curtail active binding sites and hence sequester enzymatic activity. Mathematical models demonstrate that the benefit of forming filamentous structures is to change enzyme activity rapidly (Aughey et al. 2014, Barry et al. 2014). Forming cytoophidia may simply be a strategy for storage so that the cell can harbor many molecules without releasing their activity. Storing inactive enzymes in the form of filaments can make their release adjustable to fine-tune metabolic regulation.
3. **Metabolism buffering.** The cytoophidium may serve as a metabolic stabilizer and a buffer system so that it effectively reacts to environmental changes. In the case of CTPS, when the cell needs more CTPS activity, CTPS molecules from the filament form are released into the cytoplasm to increase the concentration of free CTPS molecules. This process in turn promotes the formation of active CTPS tetramers. When the cell needs less CTPS activity, the number of active tetramers can be decreased via the reassembly of cytoophidia.
4. **Protein stabilization.** The cytoophidium could be used as a way to prolong protein life. There is evidence that the formation of filaments by drug treatment can increase the stability of the protein, preventing it from degradation by the proteasome or lysosomes.
5. **Cell proliferation.** The cytoophidium can be used as a strategy to increase the capacity of certain cells, especially fast-growing cells such as stem cells and cancer cells (Aughey et al. 2016, Tastan & Liu 2015). Like *Drosophila* neural stem cells, studies show that mouse embryonic stem cells contain abundant cytoophidia, which disassemble upon differentiation (Carcamo et al. 2011). In cancer, the formation of cytoophidia may be a sign that the cell has acquired an abnormal capacity for fast proliferation.
6. **Developmental switch.** During development, assembly and disassembly of cytoophidia can act as metabolic switches to decrease and increase enzymatic activity as required. For

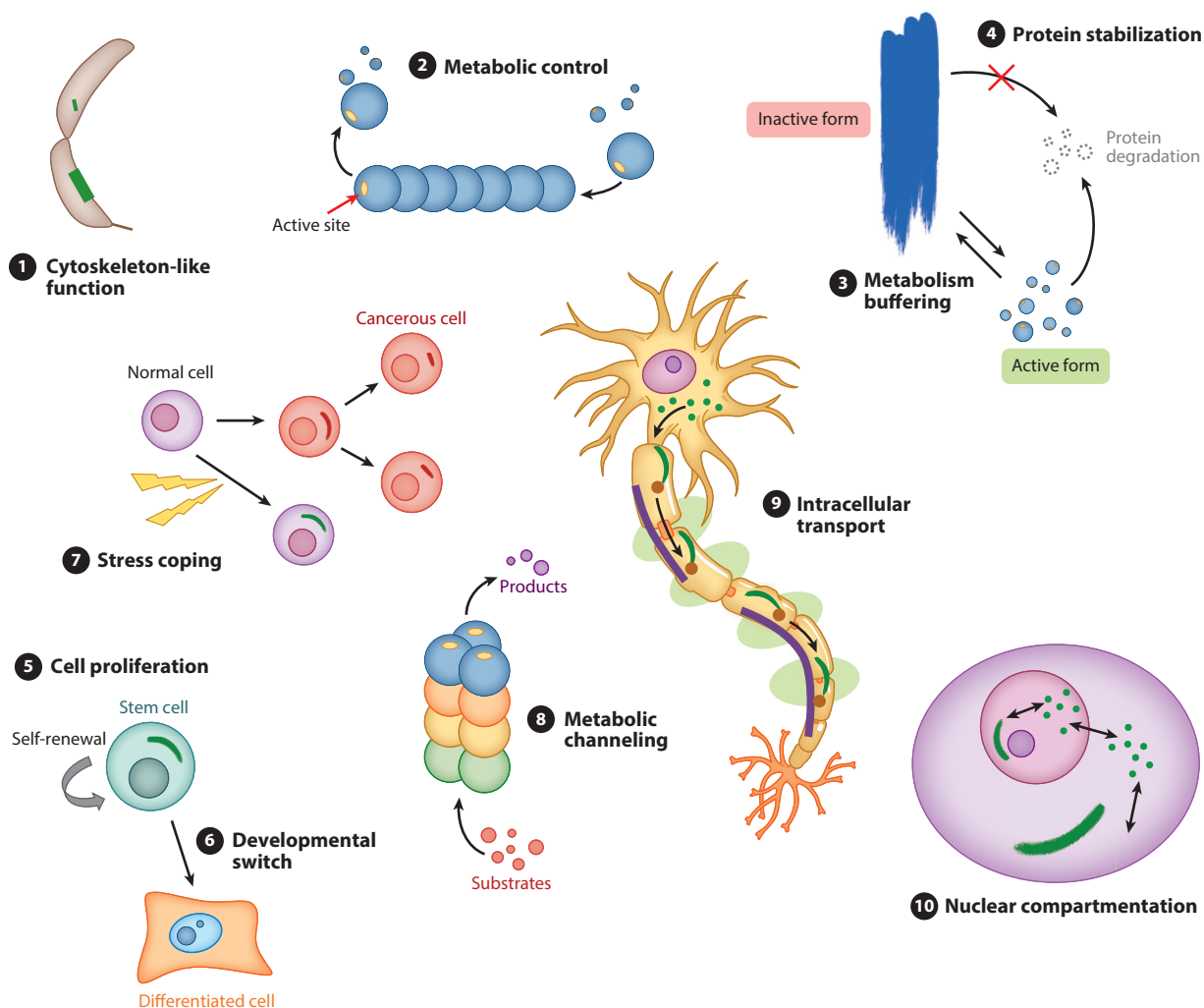


Figure 3

Speculated functions of the cytoophidium.

example, cytoophidia disassemble during the reactivation of developmentally arrested neuroblasts in *Drosophila* larvae (Aughey et al. 2014).

7. **Stress coping.** Cytoophidia can be adapted by the cell to cope with stress. Cytoophidia increase in size and frequency in cells under nutritional stress (Aughey et al. 2014). In fission yeast, heat shock makes cytoophidia fragment, whereas cold shock demolishes cytoophidium formation (J. Zhang & J.L. Liu, unpublished data).
8. **Metabolic channeling.** Cytoophidia may serve as a cooperative platform to increase the efficiency of multiple metabolic enzymes. Several enzymes may colocalize in the same filamentous structure to facilitate metabolic channeling. Increasing local concentrations of related proteins is beneficial for metabolism and other biological processes.
9. **Intracellular transport.** Packaging enzymes in the form of cytoophidia can be useful for transport. Such packaging is advantageous for long-distance transport in neurons.

Cytoophidia can be transported to synapses to change the local concentrations of certain proteins.

10. **Nuclear compartmentation.** What is the function of nuclear cytoophidia? Nuclear cytoophidia contain a pool of metabolic enzymes that are segregated from the nucleoplasm. Nuclear cytoophidia can be considered to be an extension of spatial compartmentation to increase the heterogeneity of the cell, which is fundamental for the cell to function.

Links to Disease

In 1978, Weber and coworkers found that CTPS activity in hepatomas was elevated (Williams et al. 1978). Subsequent studies demonstrated that unregulated CTP levels and increased CTPS activity are features of many forms of cancer such as leukemia, hepatomas, and colon cancer (Ellims et al. 1983; Kizaki et al. 1980; van den Berg et al. 1993, 1995; Verschuur et al. 1998, 2000a,b,c,d, 2001; Weber et al. 1980; Whelan et al. 1994; Williams et al. 1978). Importantly, knockdown of CTPS in *Drosophila* cancer models reduces tumor formation, suggesting a functional role for CTPS in cancer metabolism (Willoughby et al. 2013). A recent study showed that CTPS1 is important in lymphocyte proliferation (Martin et al. 2014). Our recent data show that the proto-oncogene Myc controls CTPS filamentation, suggesting regulation of nucleotide metabolism by Myc (Aughey et al. 2016). Cytoophidia can potentially be used as a signature of cancerous cells.

CTPS is important for brain development in *Drosophila* (Tastan & Liu 2015). The development of the neuroepithelium in *Drosophila* optic lobes coincides with that of the vertebrate cerebral cortex. *Drosophila* neuroepithelium has been used as a model system to study primary recessive microcephaly, a neurodevelopmental disorder characterized by brain size reduction at birth and by mild mental retardation. Surviving flies had smaller heads, and larvae had smaller brains with underdeveloped optic lobes. Brain size reduction in *asp* mutants is caused by defects in spindle positioning and chromosome segregation and by consequent apoptosis. Multiple CTPS mutants exhibit defects in neuroepithelium morphogenesis, resembling the phenotypes of microcephaly mutants (Tastan & Liu 2015). It would be interesting to see whether defects in CTPS and the cytoophidia contribute to microcephaly.

CTPS has also been an attractive target for drug development against viral disease (De Clercq 2001) and parasitic disease [e.g., African sleeping sickness (Fijolek et al. 2007, Hofer et al. 2001), malaria (Hendriks et al. 1998), and infectious blindness (Wylie et al. 1996)]. The cytoophidium-forming property of CTPS should be considered when one is designing antiviral or antiparasite drugs targeting CTPS.

THE IMPDH CYTOOPHIDIUM

IMPDH

Like CTPS, IMPDH is a metabolic enzyme catalyzing the rate-limiting step of de novo nucleotide biosynthesis (Hedstrom 2009, Thomas et al. 2012). IMPDH, a purine metabolic enzyme, catalyzes the oxidation of inosine-5'-monophosphate (IMP) to xanthosine-5'-monophosphate (XMP), which is then converted to guanosine-5'-monophosphate (GMP) via GMP synthase (Hedstrom 2009). As a key regulator of the intracellular guanine nucleotide pool, IMPDH is required in almost all organisms for DNA and RNA synthesis, signal transduction, and cellular growth and proliferation.

Biochemical and structural studies suggest that IMPDH can form oligomers, such as tetramers and octamers, that are composed of monomeric subunits (Labesse et al. 2013). Each monomer

contains two domains: a subdomain consisting of two repeated cystathionine beta synthetase (CBS) domains and a catalytic (β/α)₈ barrel domain with a C-terminal active site (Bateman 1997). The CBS subdomain is dispensable for the in vitro catalytic activity of IMPDH. In *E. coli*, the CBS subdomain serves as a negative transregulator of adenine nucleotide synthesis. Two isoforms, IMPDH1 and IMPDH2, exist in humans. Mutations in the CBS domain of human IMPDH1 are associated with retinitis pigmentosa (adRP10), a degenerative eye disease (Bowne et al. 2002).

The zebrafish genome has three *impdh* genes that encode three isoforms: IMPDH1a, IMPDH1b, and IMPDH2. According to a study from the Yan group, all three IMPDH genes show robust circadian expression in larval and adult zebrafish (Li et al. 2015). *impdh1a* seems to contribute to eye development and pigment synthesis. Whereas *impdh2* plays an important role in the circadian control of the cell cycle, *impdh1b* delays embryonic development, which appears to counteract the function of *impdh2*. The three *impdh* genes are likely regulated by different circadian transcription factors in zebrafish.

A *Drosophila* study demonstrates that IMPDH can bind to DNA and repress transcription (Kozhevnikova et al. 2012). In *Drosophila* S2 cells, immunostaining with antibodies against IMPDH revealed a cell cycle-dependent distribution. In G₁ phase, IMPDH localizes in the cytoplasm. However, in S and G₂ phases, IMPDH is distributed throughout both the cytoplasm and nucleus. Cytological analysis of polytene chromosomes suggests that IMPDH binds to the histone gene cluster. Chromatin immunoprecipitation in S2 cells followed by qPCR supports the idea that IMPDH binds and represses the histone genes and E2F, which encodes a transcription factor that is critical for the G₁/S transition and DNA replication (Kozhevnikova et al. 2012). Genome-wide profiling and in vitro assays show that IMPDH binds CT-rich single-stranded DNA elements. IMPDH appears to have a dual role: as a nucleotide biosynthetic enzyme to promote cell proliferation and as a transcription repressor to slow down the cell cycle.

Cytoophidia: IMPDH Versus CTPS

When treated with the inhibitor mycophenolic acid (MPA), a widely used immunosuppressant medication, IMPDH can form filaments in culture cells (Ji et al. 2006). MPA treatment can also promote filamentation of purified IMPDH (Labesse et al. 2013), suggesting that filament formation is an intrinsic property of IMPDH. Using human autoantibodies as probes, the Chan group observed distinct cytoplasmic rods (~3–10 μ m in length) and rings (~2–5 μ m in diameter) in HEp-2 cells (Carcamo et al. 2011). Accordingly, they dubbed these structures RR. In their search for the identity of RR, they ruled out actin, tubulin, and vimentin and did not see the association with centrosomes. Eventually, they revealed that antibodies against IMPDH2 and CTPS1, two key enzymes for nucleotide metabolism, could recognize the RR structures. A comprehensive review is available on the RR structures (Carcamo et al. 2014). Because RR appears to be the same filamentous structure as the cytoophidium, I refer to the RR as the cytoophidium in this review.

In addition to MPA, other drugs such as ribavirin, an adjuvant used to treat hepatitis C infection, strongly induce IMPDH cytoophidium in culture cells (Carcamo et al. 2011). Curiously, several groups have observed autoantibodies against IMPDH cytoophidium in patients infected with hepatitis C and under treatment with interferon- α and ribavirin, an IMPDH inhibitor (Calise et al. 2015, Carcamo et al. 2014, Climent et al. 2016, Keppeke et al. 2012, Novembrino et al. 2014). The autoantibodies usually appear after 6 months of treatment begins and disappear in at least half the patients after treatment is completed, suggesting that ribavirin may play a role in autoantibody production against the IMPDH cytoophidium (Keppeke et al. 2014).

At first, researchers believed that CTPS and IMPDH always colocalize with each other (Carcamo et al. 2011). However, a careful study by Keppeke et al. (2015) showed that CTPS

and IMPDH form two independent but closely related structures. Treatment with DON induces both CTPS and IMPDH cytoophidia. CTPS and IMPDH sometimes colocalize in the same structure, but at other times the CTPS cytoophidium is completely separated from the IMPDH cytoophidium. The number and length of these two types of cytoophidia are different in the same cell. In HeLa cells, the proportions of IMPDH-based, CTPS-based, and mixed IMPDH/CTPS structures are dependent on the concentration of DON used (Keppeke et al. 2015). Furthermore, the anticytoophidium autoantibodies observed in the hepatitis C-infected patients recognize only the IMPDH-based filament, which reinforces that its production is triggered by ribavirin treatment (Keppeke et al. 2015).

The finding that IMPDH and CTPS can form independent filamentous structures came as a surprise (Keppeke et al. 2015). Together with colleagues, Chang, a graduate student in the Sung group, performed a series of experiments in mammalian cells to study the relationship between IMPDH and CTPS (Chang et al. 2015). Chang et al. (2015) verified that CTPS and IMPDH form two types of cytoophidia in human HEK293T cells (**Figure 4**). Under normal culture conditions, IMPDH cytoophidia are more abundant than CTPS cytoophidia. Consistent with previous studies, DON treatment dramatically promotes the assembly of both types of cytoophidia. In most cases, CTPS and IMPDH cytoophidia appear as separate structures. However, full or partial overlap of CTPS and IMPDH cytoophidia can be observed both in the cytoplasm and in the nucleus. MPA treatment promotes the formation of IMPDH cytoophidia in 90% of cells but induces only approximately 20% of cells to form CTPS cytoophidia (Chang et al. 2015). These results indicate that assembly of the IMPDH cytoophidium is under a regulatory mechanism that is different from but interrelated with that of the CTPS cytoophidium.

The number of IMPDH cytoophidia increases when CTPS is overexpressed (Chang et al. 2015). This change could reflect changes in nucleotide synthesis. Indeed, inhibition of *de novo*

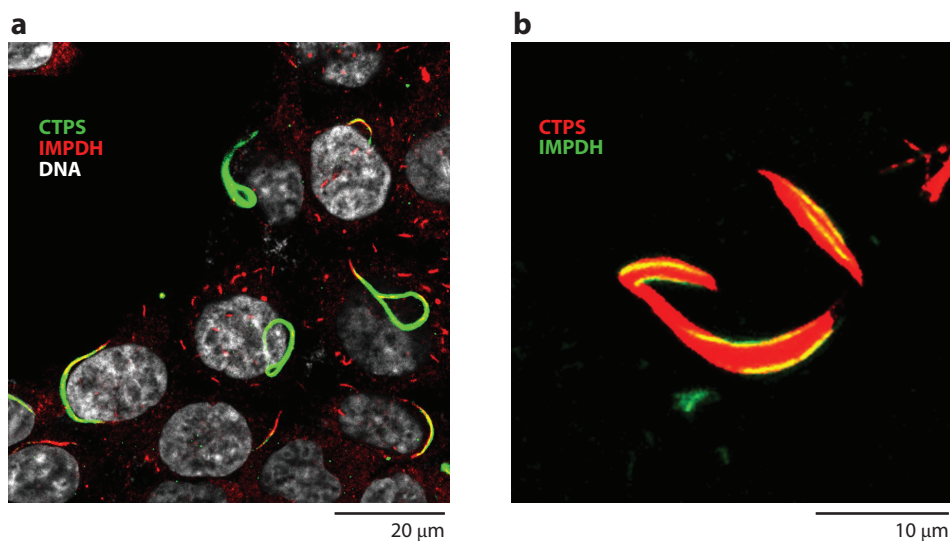


Figure 4

Cytoophidia of IMPDH and CTPS in human HEK293T cells. (a) CTPS (green) and IMPDH (red) can form independent cytoophidia. CTPS and IMPDH cytoophidia sometimes overlap (yellow). (b) Thin IMPDH cytoophidia (green) attach to the surfaces of thick CTPS cytoophidia (red). CTPS was overexpressed in both panels. Panel *a* courtesy of Chia Chun Chang and Li-Ying Sung. Panel *b* modified from Chang et al. (2015).

CTP synthesis promotes the assembly of the IMPDH cytoophidium. Whereas CTPS is sensitive to four nucleotides and catalyzes the synthesis of CTP, IMPDH is critical for GTP synthesis. The inhibition of CTP synthesis by 3'-deazauridine, an analog of uridine, activates purine nucleotide synthesis, which in turn induces the formation of IMPDH cytoophidia (Chang et al. 2015). IMPDH forms cytoophidia in most mouse BNL-CL2 cells. In these cells, inhibition of cell growth, either by serum starvation or by blocking the PI₃K-AKT-mTOR pathway, triggers disassembly of IMPDH cytoophidia (Chang et al. 2015). Moreover, IMPDH cytoophidia have been detected in mouse pancreatic islet cells, with numbers correlating with nutrient uptake by the animal (Chang et al. 2015).

Several studies suggest that the CTPS cytoophidium downregulates CTPS enzymatic activity (Aughey et al. 2014, Barry et al. 2014, Noree et al. 2014). In contrast, the assembly of the IMPDH cytoophidium appears to reflect upregulation of IMPDH activity (Chang et al. 2015). How and why do CTPS and IMPDH behave differently? How do the IMPDH and CTPS cytoophidia coordinate with each other? What are the underlying mechanisms governing the assembly of the IMPDH and CTPS cytoophidia? Many interesting questions remain to be answered.

FILAMENTATION AND METABOLIC CONTROLS

Foci Versus Filaments

The cytoophidium is distinctive in its filamentous feature. Morphologically, cytoophidia are somewhat similar to the cytoskeleton and cilia but exhibit very different forms in comparison to other organelles like lysosomes and RNA granules. Geometrically, the surface area-to-volume ratios are hugely different between filaments and foci or spherical bodies. Spherical bodies have the smallest surface area-to-volume ratio, whereas filaments have a much larger surface area-to-volume ratio. The high volume makes spherical bodies more suitable for storage, whereas the high surface area-to-volume ratio provides filaments with the advantage of being reactive to external stimuli and fine tuned. This intrinsic difference between foci and filaments may help us to understand the purpose of the filamentation of metabolic enzymes.

After screening 1,632 GFP-tagged budding yeast strains, which compose approximately 40% of the budding yeast open reading frame-GFP collection (Huh et al. 2003), the Wilhelm lab identified 9 proteins that can form filamentous structures (Noree et al. 2010). There are two CTPS proteins, Ura7p and Ura8p; both form the same structures. The Wilhelm group has shown that five representative subunits of the eIF2 and eIF2B complexes—Gcd2p (eIF2B- δ), Gcd6p (eIF2B- ϵ), Gcd7p (eIF2B- β), Gcn3p (eIF2B- α), and Sui2p (eIF2- α)—are present in the same filament (Noree et al. 2010). However, the filament containing proteins involved in the translational initiation complex is not the same as the CTPS filament. In addition to identifying filament-forming proteins, the same study identified 29 proteins that localize to foci but seem to lack the ability to form filaments (Noree et al. 2010).

Under certain conditions, filament-forming proteins can form filaments and foci in different ratios. Glutamine synthase was initially identified as a foci-forming protein that was incapable of forming filaments under standard culture conditions. However, a study by the Alberti group showed that low pH can induce glutamine synthase to form filaments, which in turn inactivate enzymatic activity (Petrovska et al. 2014). This group also demonstrated that filamentation of CTPS is sensitive to pH change, suggesting that filamentation is a general mechanism to regulate enzymatic activity.

After screening the entire collection of 4,159 GFP-tagged open reading frames in budding yeast, my group confirmed all 9 filament-forming proteins identified by Noree et al. (2010).

Including these 9 proteins, 23 proteins in total show the capability to form filaments (Shen et al. 2016). These filament-forming proteins seem to be clustered into several groups, such as translational initiation machinery and glucose and nitrogen metabolic pathways. Quantitative analyses of five glutamine-utilizing enzymes show that their sizes and abundances increase significantly when cells grow from exponential to diauxic and stationary phases (Shen et al. 2016).

Compartmentation for Metabolic Regulation

Metabolism involves cooperation of many enzymes to accomplish critical functions. Therefore, metabolic enzymes are coordinated and regulated at multiple levels. Abnormal metabolism contributes to disorders such as cancer, diabetes, and obesity. Several groups have recently demonstrated that compartmentation via filamentation of the metabolic enzymes provides a novel mechanism for regulation of metabolic processes (Aughey et al. 2014, Barry et al. 2014, Noree et al. 2014, Petrovska et al. 2014, Strohlic et al. 2014). Cytoophidium formation facilitates metabolic stabilization. Filamentation seems to be a complementary regulatory strategy for metabolic enzymes. Cytoophidium assembly not only is regulated at the transcriptional, translational, and posttranslational levels, but also responds to metabolic fluctuations caused by glutamine availability, nutritional stress, and developmental cues.

Environmental factors. Several environmental factors have been identified in the regulation of cytoophidium assembly. Treatment with DON promotes cytoophidium assembly in both *Drosophila* and human cells (Carcamo et al. 2011, Chang et al. 2015, Chen et al. 2011, Gou et al. 2014, Keppeke et al. 2015). Surprisingly, DON treatment leads to cytoophidium disassembly in *C. crescentus* (Barry et al. 2014, Ingerson-Mahar et al. 2010). This discrepancy may be due to the substrate difference between prokaryotes and eukaryotes. In prokaryotes, CTPS uses ammonia, not glutamine, as the nitrogen donor. Alternatively, the difference may lie in the timing of DON treatment used in different studies. The dependence of cytoophidium assembly on CTP levels provides another explanation (Barry et al. 2014).

Nutrient stress, such as glutamine deprivation or glucose starvation, promotes cytoophidium assembly (Aughey et al. 2014, Noree et al. 2010, Petrovska et al. 2014). In budding yeast, removing glucose from the media results in more cells having cytoophidia (Noree et al. 2010). Similarly, cytoophidium assembly increases in *Drosophila* cells cultured in phosphate-buffered saline relative to those cultured in standard culture media. Nutrient starvation leads to increased cytoophidia in tissue from *Drosophila* larvae (Aughey et al. 2014).

Developmental cues. The assembly of cytoophidia is also developmentally regulated. The postembryonic neuroblasts of the *Drosophila* central nervous system exhibit high levels of cytoplasmic (i.e., nonfilamentous) CTPS (Chen et al. 2011). Most of these neuroblasts remain in a quiescent state in early-first-instar larvae. In late-first- and early-second-instar larvae, neuroblasts exit quiescence and reenter the cell cycle. In quiescent neuroblasts, CTPS assembles into cytoophidia (Aughey et al. 2014). Upon neuroblast reactivation, cytoophidia disassemble into the diffused form. That this process seems to be regulated by the insulin signaling pathway is supported by two lines of evidence. First, whereas CTPS distributes diffusely in reactivated neuroblasts in well-fed larvae, cytoophidia are formed when the animal is in starvation. Refeeding starved larvae results in cytoophidium disassembly in neuroblasts. Second, the starvation process can be mimicked by knocking down the serine-threonine kinase *AKT1* (Aughey et al. 2014). In *Drosophila* neuroblasts, inactivation of the *AKT1* pathway promotes cytoophidium assembly.

Oligomerization interfaces. Cytoophidium assembly can be decoupled from active enzymatic sites. Point mutations in *Drosophila* CTPS show that amino acid residues at CTPS oligomer interfaces are critical for cytoophidium assembly (Aughey et al. 2014). Mutations in the tetramerization interface (*CTPS*^{G151E} and *CTPS*^{R163H}) increase cytoophidium length, whereas mutations in dimerization (*CTPS*^{V114F} and *CTPS*^{M156I}) decrease or abolish cytoophidium formation, suggesting that dimers, not tetramers, are the basic unit of the cytoophidium. Similarly, Noree et al. (2014) showed that blocking UTP-mediated tetramerization increases the frequency of cytoophidium formation in budding yeast. The length of large-sized cytoophidia is not altered when tetramerization is perturbed, suggesting that the tetramerization of CTPS affects the nucleation phase and perhaps the elongation phase, but not so much the late fusion phase or bundling phase of cytoophidium assembly.

Product inhibition. Inhibition of the end product, CTP, plays a role in the regulation of CTPS enzymatic activity (Aronow & Ullman 1987; Endrizzi et al. 2004, 2005; Kizaki et al. 1985; Long & Pardee 1967; Yang et al. 1994). A point mutation in the CTP-binding site interferes with end-product inhibition. In this mutant, CTPS forms small foci instead of large cytoophidia. These effects have been consistently observed in the fruit fly (Aughey et al. 2014), budding yeast (Noree et al. 2014), and bacteria (Barry et al. 2014). The formation of small foci suggests that end-product inhibition does not affect nucleation, the first phase of cytoophidium assembly, but is required for the later elongation and fusion phases.

CTPS senses all four major types of nucleotides. Experiments in budding yeast show that increasing CTP and ATP levels can induce nucleation, whereas GTP has no effect (Noree et al. 2010, 2014). Treatment with AMP-PNP [adenosine 5'-(β,γ -imido)triphosphate], a nonhydrolyzable analog of ATP, inhibits CTPS tetramerization, dramatically decreasing foci formation and suggesting that tetramerization is a positive factor in the nucleation phase.

A comparison of *Drosophila* CTPS isoforms identifies the N terminus of CTPS as critical for cytoophidium assembly (Azzam & Liu 2013). In addition, mutations in the ATP-binding site of CTPS increase the frequency of cytoophidium formation in budding yeast cells (Noree et al. 2014). Moreover, the allosteric GTP-binding site plays a role in both the frequency of filament formation and the length of the cytoophidium.

Structure. In vitro assays using purified *E. coli* CTPS support the idea that CTP promotes cytoophidium assembly (Barry et al. 2014). Significantly, the study by Barry et al. (2014) focused on single-stranded filaments. Thus, their results are restricted to the nucleation and early elongation phases of cytoophidium assembly.

The Barry et al. (2014) study gives impressive details of the arrangement of polymerizing CTPS. Barry et al. solve the structure of the purified *E. coli* CTPS filament by cryoelectron microscopy at 8.4-Å resolution. The X-shaped CTPS tetramers apparently rearrange the interface and are stacked on top of one another. Structure-guided mutagenesis and mathematic modeling support the hypothesis that coupling activity to polymerization enables fast and robust enzymatic regulation.

The Benefit of Filamentation

Above I summarize and speculate on the possible physiological functions of cytoophidia. Here I briefly discuss more generally the benefit of filamentation. We have learned a great deal about the mechanical roles of filamentation from studies of classical cytoskeletal filaments such as

microtubules, microfilaments, and intermediate filaments. Now we appreciate that many more proteins and enzymes can form filaments.

Several features need to be considered. First, filament formation is a very ancient phenomenon. The bacterial and human lineages have been separated for more than 3 billion years, yet their CTPS molecules form similar filamentous cytoophidia (Liu 2011). Why so? Is the cytoophidium an automatic or an accidental invention of nature? Could the cytoophidium and its kind have arisen as ancient polymers used for metabolism? The filamentation of enzymes may be a relic of the congregations from which random populations of molecules evolved metabolic activities when early life began (Dyson 1999). Both CTPS and IMPDH are critical for the synthesis of basic nucleotides and are sensitive to the concentrations of those nucleotides. Cytoophidium formation could therefore have been immensely important in the ancient RNA world, when nucleotides were tightly regulated (Gilbert 1986, Joyce 2002).

Second, filamentation has been widely adapted in various organisms. Therefore, it must be a fundamental mechanism. Does the cytoophidium serve as a basic unit in the cell? In addition, filamentation may have a secondary moonlighting function for a specific purpose in different species or different cell types.

Third, CTPS filamentation has not been abandoned over the course of natural selection, suggesting that it is beneficial for an organism's reproduction and survival. Does filamentation of enzymes make reactions more effective? Polymerization is a basic strategy for the cell. With simple combination, this process increases the variety, heterogeneity, and robustness of macromolecules within the cell. Filamentation can extend the capacity of a cell both spatially and temporally.

CONCLUDING REMARKS

Why study cytoophidia? The presence of CTPS-containing filamentous structures across diverse species suggests that cytoophidium formation is likely to have an important biological function and may represent a common regulatory strategy for the production of CTP and other nucleotides in the cell (Ingerson-Mahar et al. 2010; Liu 2010, 2011; Noree et al. 2010). Indeed, recent studies have shown that cytoophidia are dynamic structures that respond to metabolic state and external cues such as stress (Aughey et al. 2014, Barry et al. 2014, Noree et al. 2014, Petrovska et al. 2014). The compartmentation of metabolic enzymes such as CTPS and IMPDH into these filamentous structures, therefore, presents a convenient model to study the cellular mechanisms responsible for enzyme sequestration into cytoplasmic filaments and to elucidate their significance.

The study of the cytoophidium is in its infant stage. So many unanswered questions make the study of the cytoophidium very exciting. It is a new frontier of cell biology. Therefore, it is necessary to bring in expertise from other disciplines such as mathematics, biochemistry, genetics, genomics, structural biology, developmental biology, chemistry, and physics. Cutting-edge technologies will accelerate our understanding of the biology of the cytoophidium and its kind.

DISCLOSURE STATEMENT

The author is not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

I am grateful for assistance in figure preparation from Chia Chun Chang. I thank Chia Chun Chang, Li-Ying Sung, Jun Yan, and the members of the Liu lab, especially Gerson Keppeke,

for fruitful discussions. The work in my laboratory was supported by the UK Medical Research Council.

LITERATURE CITED

- An S, Kumar R, Sheets ED, Benkovic SJ. 2008. Reversible compartmentalization of de novo purine biosynthetic complexes in living cells. *Science* 320:103–6
- Anderson PM. 1983. CTP synthetase from *Escherichia coli*: an improved purification procedure and characterization of hysteretic and enzyme concentration effects on kinetic properties. *Biochemistry* 22:3285–92
- Aronow B, Ullman B. 1987. In situ regulation of mammalian CTP synthetase by allosteric inhibition. *J. Biol. Chem.* 262:5106–12
- Aughey GN, Grice SJ, Liu JL. 2016. The interplay between Myc and CTP synthase in *Drosophila*. *PLOS Genet.* 12:e1005867
- Aughey GN, Grice SJ, Shen QJ, Xu Y, Chang CC, et al. 2014. Nucleotide synthesis is regulated by cytoophidium formation during neurodevelopment and adaptive metabolism. *Biol. Open* 3:1045–56
- Azzam G, Liu JL. 2013. Only one isoform of *Drosophila melanogaster* CTP synthase forms the cytoophidium. *PLOS Genet.* 9:e1003256
- Barry RM, Bitbol AF, Lorestani A, Charles EJ, Habrian CH, et al. 2014. Large-scale filament formation inhibits the activity of CTP synthetase. *eLife* 3:e03638
- Bateman A. 1997. The structure of a domain common to archaeobacteria and the homocystinuria disease protein. *Trends Biochem. Sci.* 22:12–13
- Bearne SL, Hekmat O, Macdonnell JE. 2001. Inhibition of *Escherichia coli* CTP synthase by glutamate gamma-semialdehyde and the role of the allosteric effector GTP in glutamine hydrolysis. *Biochem. J.* 356:223–32
- Bilgen T. 2004. Metabolic evolution and the origin of life. In *Functional Metabolism: Regulation and Adaptation*, ed. KB Storey, pp. 557–82. Hoboken, NJ: Wiley-Liss
- Bowne SJ, Sullivan LS, Blanton SH, Cepko CL, Blackshaw S, et al. 2002. Mutations in the inosine monophosphate dehydrogenase 1 gene (IMPDH1) cause the RP10 form of autosomal dominant retinitis pigmentosa. *Hum. Mol. Genet.* 11:559–68
- Brangwynne CP, Eckmann CR, Courson DS, Rybarska A, Hoeghe C, et al. 2009. Germline P granules are liquid droplets that localize by controlled dissolution/condensation. *Science* 324:1729–32
- Buszczak M, Paterno S, Lighthouse D, Bachman J, Planck J, et al. 2007. The Carnegie protein trap library: a versatile tool for *Drosophila* developmental studies. *Genetics* 175:1505–31
- Calise SJ, Keppeke GD, Andrade LE, Chan EK. 2015. Anti-rods/rings: a human model of drug-induced autoantibody generation. *Front. Immunol.* 6:41
- Carcamo WC, Calise SJ, Von Muhlen CA, Satoh M, Chan EK. 2014. Molecular cell biology and immunobiology of mammalian rod/ring structures. *Int. Rev. Cell Mol. Biol.* 308:35–74
- Carcamo WC, Satoh M, Kasahara H, Terada N, Hamazaki T, et al. 2011. Induction of cytoplasmic rods and rings structures by inhibition of the CTP and GTP synthetic pathway in mammalian cells. *PLOS ONE* 6:e29690
- Chakraborty KP, Hurlbert RB. 1961. Role of glutamine in the biosynthesis of cytidine nucleotides in *Escherichia coli*. *Biochim. Biophys. Acta* 47:607–9
- Chang CC, Lin WC, Pai LM, Lee HS, Wu SC, et al. 2015. Cytoophidium assembly reflects upregulation of IMPDH activity. *J. Cell Sci.* 128:3550–55
- Chen K, Zhang J, Tastan OY, Deussen ZA, Siswick MY, Liu JL. 2011. Glutamine analogs promote cytoophidium assembly in human and *Drosophila* cells. *J. Genet. Genom.* 38:391–402
- Climont J, Morandeira F, Castellote J, Xiol J, Niubo J, et al. 2016. Clinical correlates of the “rods and rings” antinuclear antibody pattern. *Autoimmunity* 49:102–8
- De Clercq E. 2001. Vaccinia virus inhibitors as a paradigm for the chemotherapy of poxvirus infections. *Clin. Microbiol. Rev.* 14:382–97
- Dyson F. 1999. *Origins of Life*. Cambridge, UK: Cambridge Univ. Press
- Ellims PH, Gan TE, Medley G. 1983. Cytidine triphosphate synthetase activity in lymphoproliferative disorders. *Cancer Res.* 43:1432–35

- Endrizzi JA, Kim H, Anderson PM, Baldwin EP. 2004. Crystal structure of *Escherichia coli* cytidine triphosphate synthetase, a nucleotide-regulated glutamine amidotransferase/ATP-dependent amidoligase fusion protein and homologue of anticancer and antiparasitic drug targets. *Biochemistry* 43:6447–63
- Endrizzi JA, Kim H, Anderson PM, Baldwin EP. 2005. Mechanisms of product feedback regulation and drug resistance in cytidine triphosphate synthetases from the structure of a CTP-inhibited complex. *Biochemistry* 44:13491–99
- Fijolek A, Hofer A, Thelander L. 2007. Expression, purification, characterization, and in vivo targeting of trypanosome CTP synthetase for treatment of African sleeping sickness. *J. Biol. Chem.* 282:11858–65
- Gall JG. 2000. Cajal bodies: the first 100 years. *Annu. Rev. Cell Dev. Biol.* 16:273–300
- Genchev DD, Mandel P. 1976. Changes of CTP synthetase activity during postnatal rat brain development. *J. Neurosci. Res.* 2:413–18
- Gilbert W. 1986. The RNA world. *Nature* 319:618
- Goto M, Omi R, Nakagawa N, Miyahara I, Hirotsu K. 2004. Crystal structures of CTP synthetase reveal ATP, UTP, and glutamine binding sites. *Structure* 12:1413–23
- Gou KM, Chang CC, Shen QJ, Sung LY, Liu JL. 2014. CTP synthase forms cytoophidia in the cytoplasm and nucleus. *Exp. Cell Res.* 323:242–53
- Hedstrom L. 2009. IMP dehydrogenase: structure, mechanism, and inhibition. *Chem. Rev.* 109:2903–28
- Hendriks EF, O’Sullivan WJ, Stewart TS. 1998. Molecular cloning and characterization of the *Plasmodium falciparum* cytidine triphosphate synthetase gene. *Biochim. Biophys. Acta* 1399:213–18
- Higgins MJ, Loisele D, Haystead TA, Graves LM. 2008. Human cytidine triphosphate synthetase 1 interacting proteins. *Nucleosides Nucleotides Nucleic Acids* 27:850–57
- Hofer A, Steverding D, Chabes A, Brun R, Thelander L. 2001. *Trypanosoma brucei* CTP synthetase: a target for the treatment of African sleeping sickness. *PNAS* 98:6412–16
- Huh WK, Falvo JV, Gerke LC, Carroll AS, Howson RW, et al. 2003. Global analysis of protein localization in budding yeast. *Nature* 425:686–91
- Hung V, Zou P, Rhee HW, Udeshi ND, Cracan V, et al. 2014. Proteomic mapping of the human mitochondrial intermembrane space in live cells via ratiometric APEX tagging. *Mol. Cell* 55:332–41
- Hyman AA, Weber CA, Julicher F. 2014. Liquid-liquid phase separation in biology. *Annu. Rev. Cell Dev. Biol.* 30:39–58
- Ingerson-Mahar M, Briegel A, Werner JN, Jensen GJ, Gitai Z. 2010. The metabolic enzyme CTP synthase forms cytoskeletal filaments. *Nat. Cell Biol.* 12:739–46
- Ji Y, Gu J, Makhov AM, Griffith JD, Mitchell BS. 2006. Regulation of the interaction of inosine monophosphate dehydrogenase with mycophenolic acid by GTP. *J. Biol. Chem.* 281:206–12
- Joyce GF. 2002. The antiquity of RNA-based evolution. *Nature* 418:214–21
- Kammen HO, Hurlbert RB. 1958. Amination of uridine nucleotides to cytidine nucleotides by soluble mammalian enzymes; role of glutamine and guanosine nucleotides. *Biochim. Biophys. Acta* 30:195–96
- Kammen HO, Hurlbert RB. 1959. The formation of cytidine nucleotides and RNA cytosine from orotic acid by the Novikoff tumor in vitro. *Cancer Res.* 19:654–63
- Keppeke GD, Calise SJ, Chan EK, Andrade LE. 2015. Assembly of IMPDH2-based, CTPS-based, and mixed rod/ring structures is dependent on cell type and conditions of induction. *J. Genet. Genom.* 42:287–99
- Keppeke GD, Nunes E, Ferraz ML, Silva EA, Granato C, et al. 2012. Longitudinal study of a human drug-induced model of autoantibody to cytoplasmic rods/rings following HCV therapy with ribavirin and interferon-alpha. *PLOS ONE* 7:e45392
- Keppeke GD, Satoh M, Ferraz ML, Chan EK, Andrade LE. 2014. Temporal evolution of human autoantibody response to cytoplasmic rods and rings structure during anti-HCV therapy with ribavirin and interferon-alpha. *Immunol. Res.* 60:38–49
- Kizaki H, Ohsaka F, Sakurada T. 1985. CTP synthetase from Ehrlich ascites tumor cells. Subunit stoichiometry and regulation of activity. *Biochim. Biophys. Acta* 829:34–43
- Kizaki H, Williams JC, Morris HP, Weber G. 1980. Increased cytidine 5'-triphosphate synthetase activity in rat and human tumors. *Cancer Res.* 40:3921–27
- Kozhevnikova EN, Van Der Knaap JA, Pindyurin AV, Ozgur Z, Van Ijcken WF, et al. 2012. Metabolic enzyme IMPDH is also a transcription factor regulated by cellular state. *Mol. Cell* 47:133–39

- Labesse G, Alexandre T, Vaupre L, Salard-Arnaud I, Him JL, et al. 2013. MgATP regulates allostery and fiber formation in IMPDHs. *Structure* 21:975–85
- Lam SS, Martell JD, Kamer KJ, Deerinck TJ, Ellisman MH, et al. 2015. Directed evolution of APEX2 for electron microscopy and proximity labeling. *Nat. Methods* 12:51–54
- Lee L, Davies SE, Liu JL. 2009. The spinal muscular atrophy protein SMN affects *Drosophila* germline nuclear organization through the U body–P body pathway. *Dev. Biol.* 332:142–55
- Levitzki A, Koshland DE Jr. 1969. Negative cooperativity in regulatory enzymes. *PNAS* 62:1121–28
- Levitzki A, Koshland DE Jr. 1970. Ligand-induced association-dissociation as a means for enzyme purification. *Biochim. Biophys. Acta* 206:473–75
- Levitzki A, Koshland DE Jr. 1971. Cytidine triphosphate synthetase. Covalent intermediates and mechanisms of action. *Biochemistry* 10:3365–71
- Levitzki A, Koshland DE Jr. 1972a. Ligand-induced dimer-to-tetramer transformation in cytosine triphosphate synthetase. *Biochemistry* 11:247–53
- Levitzki A, Koshland DE Jr. 1972b. Role of an allosteric effector. Guanosine triphosphate activation in cytosine triphosphate synthetase. *Biochemistry* 11:241–46
- Levitzki A, Stallcup WB, Koshland DE Jr. 1971. Half-of-the-sites reactivity and the conformational states of cytidine triphosphate synthetase. *Biochemistry* 10:3371–78
- Lewis DA, Villafranca JJ. 1989. Investigation of the mechanism of CTP synthetase using rapid quench and isotope partitioning methods. *Biochemistry* 28:8454–59
- Li Y, Li G, Gorling B, Luy B, Du J, Yan J. 2015. Integrative analysis of circadian transcriptome and metabolic network reveals the role of de novo purine synthesis in circadian control of cell cycle. *PLOS Comput. Biol.* 11:e1004086
- Li Z, Jensen GJ. 2009. Electron cryotomography: a new view into microbial ultrastructure. *Curr. Opin. Microbiol.* 12:333–40
- Lieberman I. 1955. Enzymatic amination of uridine triphosphate to cytidine triphosphate. *J. Am. Chem. Soc.* 77:2661–62
- Lieberman I. 1956. Enzymatic amination of uridine triphosphate to cytidine triphosphate. *J. Biol. Chem.* 222:765–75
- Liu JL. 2010. Intracellular compartmentation of CTP synthase in *Drosophila*. *J. Genet. Genom.* 37:281–96
- Liu JL. 2011. The enigmatic cytoophidium: compartmentation of CTP synthase via filament formation. *BioEssays* 33:159–64
- Liu JL, Buszczak M, Gall JG. 2006a. Nuclear bodies in the *Drosophila* germinal vesicle. *Chromosome Res.* 14:465–75
- Liu JL, Gall JG. 2007. U bodies are cytoplasmic structures that contain uridine-rich small nuclear ribonucleoproteins and associate with P bodies. *PNAS* 104:11655–59
- Liu JL, Murphy C, Buszczak M, Clatterbuck S, Goodman R, Gall JG. 2006b. The *Drosophila melanogaster* Cajal body. *J. Cell Biol.* 172:875–84
- Liu YC, Li F, Handler J, Huang CR, Xiang Y, et al. 2008. Global regulation of nucleotide biosynthetic genes by c-Myc. *PLOS ONE* 3:e2722
- Long CW, Levitzki A, Koshland DE Jr. 1970. The subunit structure and subunit interactions of cytidine triphosphate synthetase. *J. Biol. Chem.* 245:80–87
- Long CW, Pardee AB. 1967. Cytidine triphosphate synthetase of *Escherichia coli* B. I. Purification and kinetics. *J. Biol. Chem.* 242:4715–21
- Martell JD, Deerinck TJ, Sancak Y, Poulos TL, Mootha VK, et al. 2012. Engineered ascorbate peroxidase as a genetically encoded reporter for electron microscopy. *Nat. Biotechnol.* 30:1143–48
- Martin E, Palmic N, Sanquer S, Lenoir C, Hauck F, et al. 2014. CTP synthase 1 deficiency in humans reveals its central role in lymphocyte proliferation. *Nature* 510:288–92
- Narayanaswamy R, Levy M, Tsechansky M, Stovall GM, O’Connell JD, et al. 2009. Widespread reorganization of metabolic enzymes into reversible assemblies upon nutrient starvation. *PNAS* 106:10147–52
- Noree C, Monfort E, Shiau AK, Wilhelm JE. 2014. Common regulatory control of CTP synthase enzyme activity and filament formation. *Mol. Biol. Cell* 25:2282–90
- Noree C, Sato BK, Broyer RM, Wilhelm JE. 2010. Identification of novel filament-forming proteins in *Saccharomyces cerevisiae* and *Drosophila melanogaster*. *J. Cell Biol.* 190:541–51

- Novembrino C, Aghemo A, Ferraris Fusarini C, Maiavacca R, Matinato C, et al. 2014. Interferon-ribavirin therapy induces serum antibodies determining ‘rods and rings’ pattern in hepatitis C patients. *J. Viral Hepat.* 21:944–49
- O’Connell JD, Zhao A, Ellington AD, Marcotte EM. 2012. Dynamic reorganization of metabolic enzymes into intracellular bodies. *Annu. Rev. Cell Dev. Biol.* 28:89–111
- Ovadi J, Saks V. 2004. On the origin of intracellular compartmentation and organized metabolic systems. *Mol. Cell. Biochem.* 256–257:5–12
- Pappas A, Yang WL, Park TS, Carman GM. 1998. Nucleotide-dependent tetramerization of CTP synthetase from *Saccharomyces cerevisiae*. *J. Biol. Chem.* 273:15954–60
- Petrovska I, Nuske E, Munder MC, Kulasegaran G, Malinowska L, et al. 2014. Filament formation by metabolic enzymes is a specific adaptation to an advanced state of cellular starvation. *eLife* 3:e02409
- Rhee HW, Zou P, Udeshi ND, Martell JD, Mootha VK, et al. 2013. Proteomic mapping of mitochondria in living cells via spatially restricted enzymatic tagging. *Science* 339:1328–31
- Robertson JG. 1995. Determination of subunit dissociation constants in native and inactivated CTP synthetase by sedimentation equilibrium. *Biochemistry* 34:7533–41
- Scheit KH, Linke HJ. 1982. Substrate specificity of CTP synthetase from *Escherichia coli*. *Eur. J. Biochem.* 126:57–60
- Shen QJ, Kassim H, Huang Y, Li H, Zhang J, et al. 2016. Filamentation of metabolic enzymes in *Saccharomyces cerevisiae*. *J. Genet. Genom.* 43:393–404
- Sheth U, Parker R. 2006. Targeting of aberrant mRNAs to cytoplasmic processing bodies. *Cell* 125:1095–109
- Sitte P. 1980. General principles of cellular compartmentation. In *Cell Compartmentation and Metabolic Channeling*, ed. L Nover, F Lynen, K Mothes, pp. 17–32. Amsterdam: Elsevier
- Strochlic TI, Stavrides KP, Thomas SV, Nicolas E, O’Reilly AM, Peterson JR. 2014. Ack kinase regulates CTP synthase filaments during *Drosophila* oogenesis. *EMBO Rep.* 15:1184–91
- Tastan OY, Liu JL. 2015. CTP synthase is required for optic lobe homeostasis in *Drosophila*. *J. Genet. Genom.* 42:261–74
- Thomas EC, Gunter JH, Webster JA, Schieber NL, Oorschot V, et al. 2012. Different characteristics and nucleotide binding properties of inosine monophosphate dehydrogenase (IMPDH) isoforms. *PLOS ONE* 7:e51096
- Van Den Berg AA, Van Lenthe H, Busch S, De Korte D, Roos D, et al. 1993. Evidence for transformation-related increase in CTP synthetase activity in situ in human lymphoblastic leukemia. *Eur. J. Biochem.* 216:161–67
- Van Den Berg AA, Van Lenthe H, Kipp JB, De Korte D, Van Kuilenburg AB, Van Gennip AH. 1995. Cytidine triphosphate (CTP) synthetase activity during cell cycle progression in normal and malignant T-lymphocytic cells. *Eur. J. Cancer* 31A:108–12
- Verschuur AC, Brinkman J, Van Gennip AH, Leen R, Vet RJ, et al. 2001. Cyclopentenyl cytosine induces apoptosis and increases cytarabine-induced apoptosis in a T-lymphoblastic leukemic cell-line. *Leuk. Res.* 25:891–900
- Verschuur AC, Van Gennip AH, Brinkman J, Voute PA, Van Kuilenburg AB. 2000a. Cyclopentenyl cytosine induces apoptosis and secondary necrosis in a T-lymphoblastic leukemic cell-line. *Adv. Exp. Med. Biol.* 486:319–25
- Verschuur AC, Van Gennip AH, Leen R, Meinsma R, Voute PA, Van Kuilenburg AB. 2000b. In vitro inhibition of cytidine triphosphate synthetase activity by cyclopentenyl cytosine in paediatric acute lymphocytic leukaemia. *Br. J. Haematol.* 110:161–69
- Verschuur AC, Van Gennip AH, Leen R, Muller EJ, Elzinga L, et al. 2000c. Cyclopentenyl cytosine inhibits cytidine triphosphate synthetase in paediatric acute non-lymphocytic leukaemia: a promising target for chemotherapy. *Eur. J. Cancer* 36:627–35
- Verschuur AC, Van Gennip AH, Leen R, Voute PA, Van Kuilenburg AB. 2000d. Cyclopentenyl cytosine increases the phosphorylation and incorporation into DNA of arabinofur-anosyl cytosine in a myeloid leukemic cell-line. *Adv. Exp. Med. Biol.* 486:311–17
- Verschuur AC, Van Gennip AH, Muller EJ, Voute PA, Van Kuilenburg AB. 1998. Increased activity of cytidine triphosphate synthetase in pediatric acute lymphoblastic leukemia. *Adv. Exp. Med. Biol.* 431:667–71

- Von Der Saal W, Anderson PM, Villafranca JJ. 1985. Mechanistic investigations of *Escherichia coli* cytidine-5'-triphosphate synthetase. Detection of an intermediate by positional isotope exchange experiments. *J. Biol. Chem.* 260:14993–97
- Wang PY, Lin WC, Tsai YC, Cheng ML, Lin YH, et al. 2015. Regulation of CTP synthase filament formation during DNA endoreplication in *Drosophila*. *Genetics* 201:1511–23
- Weber G, Lui MS, Takeda, E, Denton JE. 1980. Enzymology of human colon tumors. *Life Sci.* 27:793–99
- Whelan J, Smith T, Phear G, Rohatiner A, Lister A, Meuth M. 1994. Resistance to cytosine arabinoside in acute leukemia: the significance of mutations in CTP synthetase. *Leukemia* 8:264–65
- Williams JC, Kizaki H, Weber G, Morris HP. 1978. Increased CTP synthetase activity in cancer cells. *Nature* 271:71–73
- Willoughby LF, Schlosser T, Manning SA, Parisot JP, Street IP, et al. 2013. An in vivo large-scale chemical screening platform using *Drosophila* for anti-cancer drug discovery. *Dis. Model. Mech.* 6:521–29
- Wu CH, Sahoo D, Arvanitis C, Bradon N, Dill DL, Felsher DW. 2008. Combined analysis of murine and human microarrays and ChIP analysis reveals genes associated with the ability of MYC to maintain tumorigenesis. *PLOS Genet.* 4:e1000090
- Wylie JL, Berry JD, McClarty G. 1996. *Chlamydia trachomatis* CTP synthetase: molecular characterization and developmental regulation of expression. *Mol. Microbiol.* 22:631–42
- Yang WL, Bruno ME, Carman GM. 1996. Regulation of yeast CTP synthetase activity by protein kinase C. *J. Biol. Chem.* 271:11113–19
- Yang WL, Carman GM. 1995. Phosphorylation of CTP synthetase from *Saccharomyces cerevisiae* by protein kinase C. *J. Biol. Chem.* 270:14983–88
- Yang WL, Carman GM. 1996. Phosphorylation and regulation of CTP synthetase from *Saccharomyces cerevisiae* by protein kinase A. *J. Biol. Chem.* 271:28777–83
- Yang WL, McDonough VM, Ozier-Kalogeropoulos O, Adeline MT, Flocco MT, Carman GM. 1994. Purification and characterization of CTP synthetase, the product of the URA7 gene in *Saccharomyces cerevisiae*. *Biochemistry* 33:10785–93
- Zhang J, Hulme L, Liu JL. 2014. Asymmetric inheritance of cytoophidia in *Schizosaccharomyces pombe*. *Biol. Open* 3:1092–97



Contents

The Heidelberg Screen for Pattern Mutants of <i>Drosophila</i> : A Personal Account <i>Eric Wieschaus and Christiane Nüsslein-Volhard</i>	1
Tissue and Organ Initiation in the Plant Embryo: A First Time for Everything <i>Joakim Palovaara, Thijs de Zeeuw, and Dolf Weijers</i>	47
Transcriptional Control of Developmental Cell Behaviors <i>Yelena Bernadskaya and Lionel Christiaen</i>	77
Genotypes, Networks, Phenotypes: Moving Toward Plant Systems Genetics <i>Takebiko Ogura and Wolfgang Busch</i>	103
Neurogenesis and Gliogenesis in Nervous System Plasticity and Repair <i>Jonas Frisén</i>	127
Phosphoinositides in Control of Membrane Dynamics <i>Kay O. Schink, Kia-Wee Tan, and Harald Stenmark</i>	143
Cytoplasmic Streaming in the <i>Drosophila</i> Oocyte <i>Margot E. Quinlan</i>	173
Cargo Capture and Bulk Flow in the Early Secretory Pathway <i>Charles Barlowe and Ari Helenius</i>	197
The Lysosome as a Regulatory Hub <i>Rushika M. Perera and Roberto Zoncu</i>	223
TFEB and TFE3: Linking Lysosomes to Cellular Adaptation to Stress <i>Nina Raben and Rosa Puertollano</i>	255
Endoplasmic Reticulum–Plasma Membrane Associations: Structures and Functions <i>Alessandra Gallo, Christian Vannier, and Thierry Galli</i>	279
Signaling and Polarized Communication Across the T Cell Immunological Synapse <i>Michael L. Dustin and Kaushik Choudhuri</i>	303

TCR Signal Strength and T Cell Development <i>Nicholas R.J. Gascoigne, Vasily Rybakin, Oreste Acuto, and Joanna Brzostek</i>	327
The Cytoophidium and Its Kind: Filamentation and Compartmentation of Metabolic Enzymes <i>Ji-Long Liu</i>	349
How Bacteria Subvert Animal Cell Structure and Function <i>Alyssa Jimenez, Didi Chen, and Neal M. Alto</i>	373
Metabolism and the Control of Cell Fate Decisions and Stem Cell Renewal <i>Kyoko Ito and Keisuke Ito</i>	399
Cell Competition: Mechanisms and Physiological Roles <i>Cristina Clavería and Miguel Torres</i>	411
Functions and Regulation of Programmed Cell Death in Plant Development <i>Anna Daneva, Zhen Gao, Matthias Van Durme, and Moritz K. Nowack</i>	441
Focal Adhesion–Independent Cell Migration <i>Ewa K. Paluch, Irene M. Aspalter, and Michael Sixt</i>	469
Plasticity of Cell Migration In Vivo and In Silico <i>Veronika te Boekhorst, Luigi Preziosi, and Peter Friedl</i>	491
Mechanical Control of Epithelial-to-Mesenchymal Transitions in Development and Cancer <i>Laralynne Przybyla, Jonathon M. Muncie, and Valerie M. Weaver</i>	527
Cellular and Molecular Mechanisms of MT1-MMP-Dependent Cancer Cell Invasion <i>Antonio Castro-Castro, Valentina Marchesin, Pedro Monteiro, Catalina Lodillinsky, Carine Rossé, and Philippe Chavrier</i>	555
Structural Perspectives on Axon Guidance <i>Elena Seiradake, E. Yvonne Jones, and Rüdiger Klein</i>	577
The Role of Adipocytes in Tissue Regeneration and Stem Cell Niches <i>Brett Shook, Guillermo Rivera Gonzalez, Sarah Ebmeier, Gabriella Grisotti, Rachel Zwick, and Valerie Horsley</i>	609
Hemodynamic Control of Endothelial Cell Fates in Development <i>Guillermo García-Cardena and Bendix R. Slegtenhorst</i>	633
Regulation of Hematopoiesis and Osteogenesis by Blood Vessel–Derived Signals <i>Saravana K. Ramasamy, Anjali P. Kusumbe, Tomer Itkin, Shiri Gur-Cohen, Tsvee Lapidot, and Ralf H. Adams</i>	649

Lymphangiogenesis: Origin, Specification, and Cell Fate
Determination
Noelia Escobedo and Guillermo Oliver 677

Emergence and Evolution of Secondary Lymphoid Organs
Harold R. Neely and Martin F. Flajnik 693

Chemical Principles in Tissue Clearing and Staining Protocols for
Whole-Body Cell Profiling
*Kazuki Tainaka, Akibiro Kuno, Shimpei I. Kubota, Tatzya Murakami,
and Hiroki R. Ueda* 713

Indexes

Cumulative Index of Contributing Authors, Volumes 28–32 743

Cumulative Index of Article Titles, Volumes 28–32 747

Errata

An online log of corrections to *Annual Review of Cell and Developmental Biology* articles
may be found at <http://www.annualreviews.org/errata/cellbio>

Annu. Rev. Cell Dev. Biol. 2016.32:349-372. Downloaded from www.annualreviews.org
Access provided by Shanghai Tech University on 02/11/17. For personal use only.