What can flies tell us about zinc homeostasis?

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Abstract

Zinc is an essential micronutrient for all organisms. For multicellular organisms, zinc uptake, storage, distribution and export are tightly regulated at both cellular and organismal levels, to cope with the multiple requirements versus the toxicity of the metal ion. During the past decade, the fruit fly *Drosophila melanogaster* has become an important model organism for the elucidation of metazoan zinc homeostasis. This review describes our current knowledge of various zinc transporters in *Drosophila*, with an emphasis on the process of dietary zinc uptake in the fly. We also discuss how *Drosophila* was used as a model to facilitate our understanding of the role of zinc in neurodegenerative diseases.

Keywords

Drosophila, zinc, homeostasis, gut, neurodegeneration

Introduction

Zinc plays a catalytic or structural role in many enzymes and numerous proteins, and accordingly, contributes to a variety of fundamental biological processes¹⁻³. Zinc dyshomeostasis could lead to various defects in multiple biological progresses. Although intensive study of zinc transporters at the cellular level has been performed in recent decades, their physiological functions on the maintenance of zinc homeostasis at the organismal level is less well characterized.

Our current knowledge of zinc metabolism largely comes from mammalian and microbial research. Questions at the organismal level have to be addressed properly with multi-cellular organisms. In terms of rodents, one limitation so far is that not all zinc transporters in mice have been targeted for mutagenesis and for those that have been targeted, mutations were usually generated ubiquitously rather than tissue-specifically, making it difficult to pinpoint their specific roles in a specific tissue or biological process. Another limit is likely functional redundancies among the zinc transporters, making functional analysis sometimes difficult unless various mutations are combined together, which can be time and financially costly.

As a complement to these approaches, *Drosophila* has been used fruitfully for

metal studies. For example, more than 50 years ago copper study in *Drosophila* was performed⁴. *Drosophila* also helped elucidate the molecular action of the environmental toxin aluminum⁵. Although studying zinc in *Drosophila* is a relatively recent event, large strides have already been made. This review mainly summarizes our current understanding of *Drosophila* zinc homeostasis and its relevance to diseases especially neurodegenerations.

1. *Drosophila melanogaster*, a model organism to study zinc homeostasis

The fruit fly *D. melanogaster* represents one of the most important model organisms in genetics and developmental biology. *D. melanogaster* is a 2-3 mm long organism which is often found around grapes, rotten bananas and other fruits. Many characteristics including its small size, short life cycle (about 12 days), large amount of offsprings, ease of culturing, and the availability of many mutant strains and techniques make the fruit fly an ideal model for genetic studies. The completion of genome sequencing^{6,7} and generation of a large number of overexpression or RNAi fly lines further facilitate the use of *Drosophila* in biological research.

Drosophila contains approximately 13600 genes, located in only four chromosomes. It has been shown that about 39% of human genes have orthologues⁸, and 70% of human disease causing genes have homologues in the fly^{9,10}. The intensive use of *Drosophila* as a model for developmental and genetic investigations has produced a broad range of genetic and molecular approaches that can be readily adapted for the research of zinc homeostasis.

2. ZIP and ZnT transporters in *Drosophila*

Mammalian zinc transporters largely fall into two conserved families, the ZnT (SLC30) family and the ZIP (SLC39) family. Members of the ZnT family generally function in zinc efflux or sequestration into organelles/vesicles, and those of the ZIP family mediate zinc influx from the extracellular medium or vesicular organelles into the cytoplasm^{3,11-14}. As shown in Table 1, seven members of the ZnT family and ten members of the ZIP family are found in the *Drosophila* proteome, displaying homology to mammalian zinc transporters to potentially control zinc homeostasis in *Drosophila*. *Drosophila* zinc transporters are identified in multiple ways and named by different principles due to historical reasons. In order to be unified, they are annotated by CG annotation in this review, and frequently noted by their relevance to mammalian counterparts to facilitate the reading for people outside of the field. All published names for each transporter are presented in Table 1.

2.1. Expression pattern and subcellular localization

The endogenous expression pattern or subcellular localization of some zinc transporters have been analyzed in *Drosophila*, including CG17723, CG3994, CG9428 and CG7816. CG17723, a close homologue of ZnT1, is most prominent in the plasma membrane of midgut constrictions, malpighian tubules and is also found in the testis and salivary glands¹⁵. CG3994, similar to a set of intracellular ZnTs such as ZnT2, 3 or 8 at the amino acid level, is surprisingly found on the plasma membrane of

malpighian tubules¹⁶. CG9428, similar to ZIP1, 2 and 3, mainly expresses in the midgut, and is also present in the trachea and testis; it is also located on the plasma membrane¹⁴. CG7816, or dZIP13, is an intracellular iron transporter located on the secretory pathway, but its tissue and developmental expression patterns have not been characterized in detail¹⁷. Unavailability of antibodies for the other zinc transporters at present limits a direct characterization of their expression patterns and subcellular localizations.

A fusion of *Drosophila* ZIP and ZnT with the enhanced green fluorescent protein (eGFP), combined with by overexpression in the larval salivary glands, has allowed a fast test of the intracellular localizations of many zinc transporters¹⁸. Among these transporters, only CG17723, CG6817 and CG9428 were found to locate on the plasma membrane, CG11163 and CG10449 solely intracellular, and all the others were both on the plasma membrane and intracellular¹⁸. This study provides some valuable information for the study of *Drosophila* zinc transporters, but not without caveats. Protein fusion and overexpression could result in mislocalizations. In addition, different localizations of the same protein may occur in different tissues.

At the RNA level, the expression pattern of most genes could be found in some public *Drosophila* gene expression databases such as the Berkeley *Drosophila* Gene Expression Pattern Database (BDGP)^{19,20} and the FlyAtlas Expression Data²¹⁻²². We have summarized the RNA expression data of other zinc transporters not mentioned above in table 1 according to BDGP and FlyAtlas.

2.2. Natural mutants of zinc transporters in *Drosophila*

Functions of genes can best be revealed by their loss-of-function mutations. Interestingly there are two natural mutants due to zinc transporter mutations. Both of them display remarkable phenotypes.

a) *fear of intimacy (foi)*

Drosophila foi (encoded by CG6817) is a ZIP family member initially identified in a large-scale screen for mutations affecting gonad formation in *Drosophila*²³, and later found also required for the formation of the tracheal branch fusion during tracheal development^{24,25}. *foi* mutant embryos fail to form the characteristic round shape gonads that are composed of intimately associated germ cells, instead, these cells remain only loosely aligned in a line^{23,25}. *foi* mutants die at the end of embryogenesis with obvious abnormalities in the developing tracheal system, but no general defects were observed in a variety of other tissues analyzed including the nervous system, midgut, musculature and embryonic cuticle. The tracheal defect of *foi* may underlie the lethality of *foi* mutant.

The protein encoded by *foi* is localized to the cell plasma membrane and the closest human homologues of FOI may be hZIP6 and hZIP10. When expressed in both yeast and mammalian cells, FOI functions as a zinc transporter^{24,25}. Zinc transport activity of *foi* is essential for the expression of the hemophilic cell-adhesion molecule *Drosophila* E-cadherin (DE-cadherin), by regulating the accumulation of DE-cadherin in the gonad at the post-transcriptional level. This process is essential for proper

gonad morphogenesis²⁶.

b) *Catecholamines up (Catsup)*

Catsup (encoded by CG10449) is another ZIP family member essential for development in *Drosophila*. *Catsup* was first identified as a negative regulator of tyrosine hydroxylase (TH, the rate-limiting enzyme for dopamine biosynthesis) activity in *D. melanogaster*²⁷. Elevated TH activity due to *Catsup* mutations leads to abnormally high levels of catecholamines, resulting in a set of defects such as incomplete cuticle formation, melanotic pseudotumors, female sterility, tracheal development abnormality and lethality^{27,28}. Besides, the mutations are dominantly neuroprotective against paraquat exposure likely because of the elevated dopamine level²⁹. The exact mechanism for the negative regulation of TH activity by *Catsup* remains unelucidated yet. Janis M. O'Donnell's lab reported that *Catsup* physically associates with TH and GTPCH (the rate-limiting enzyme for the biosynthesis of tetrahydrobiopterin (BH4, a cofactor of TH) and proposed this interaction may negatively regulate their activities, and simultaneously functions in the regulation of synaptic vesicle loading and release of dopamine^{29,30}.

Phylogenetic analysis indicated that the protein encoded by *Catsup* is a close and likely the sole *Drosophila* homolog of mammalian ZIP7^{25,31,32}. *Catsup* mutations lead to abnormal accumulation of membrane proteins in the secretory compartment and is accompanied by induction of the ER stress response and increased levels of apoptosis, revealing *Catsup* plays a general role in the maintenance of ER/Golgi function and protein trafficking³².

2.3. Phenotypes of other zinc transporters when ubiquitously knocked down

Given the existence of a plethora of potential zinc importers and exporters in *Drosophila*, with different temporal and spatial expression specificities, as well as likely different metal affinities and transporting abilities, their roles in zinc homeostasis and other physiological functions may much vary.

Since there are only two known natural mutants of zinc transporters, in order to identify the functions of other zinc transport genes that are important in *Drosophila*, we performed a small-scale screen with the RNAi lines of 15 of the 17 putative zinc transport genes in *Drosophila*. Although subtle phenotypes remain to be characterized, some obvious defects due to ubiquitous loss of function of these zinc transporters are summarized here. Their gut-specific functions will be analyzed in a later section under Zinc absorption in *Drosophila*.

When ubiquitously knocked down, five transporters displayed zinc-dependent phenotypes, indicating important roles played by these zinc transporters in zinc homeostasis. CG17723 (dZnT1), CG5130 or CG9430 knockdown is sensitive on zinc-depleted diet (table 1). CG3994 knockdown exhibited sensitivity to zinc supplement in the food¹⁴⁻¹⁶. CG6898 knockdown is resistant to high dietary zinc, suggesting a partial redundant function with CG9428 and CG9430 in the dietary zinc absorption in *Drosophila* (further discuss in later section)³³. Seven members including CG31860, CG6672, CG8632, CG4334, CG6817 (*foi*), CG10449 (*Catsup*) and

CG7816 appear to be important for development; ubiquitous knockdown of them lead to development arrest at different stages (table 1). Among them only CG4334 knockdown could be rescued by zinc depletion in the food. Surprisingly CG7816 (dZIP13) knockdown could be rescued by iron supplement in the diet, suggesting its involvement in iron metabolism (discussed more later in Dietary zinc absorption in *Drosophila*) (table 1)¹⁷.

3. Transient receptor potential melastatin (TRPM)

Transient receptor potential (TRP) proteins are a large family of cation channels with a variety of functions³⁴, and TRPM is a subfamily of it. The *Drosophila* genome is reported to encode a single TRPM gene (dTRPM)^{35,36}. Padinjat Raghu's group reported that *dTRPM* deficiency leads to reduced zinc levels and decreased cell size as well as defects in the mitochondrial structure and function, resulting in reduction of larval growth. These defects could be rescued by zinc supplementation in the food. They postulated that regulation of Zn²⁺ homeostasis through dTRPM to a subcellular compartment may be important to mediate class I PI3K-regulated cell growth³⁷.

Craig Montell's group reported that dTRPM mutations resulted in shortening of the malpighian tubules and the mutants underwent complete developmental arrest as prepupae; these mutants are, however, sensitive to elevated dietary Mg²⁺ levels. They found that dTRPM was located on the apical membrane of malpighian tubule cells and dTRPM mutants had increased Mg²⁺ and decreased Ca²⁺ in the circulating lymph. They concluded that dTRPM participates in Mg²⁺ removal from the hemolymph via the malpighian tubules³⁸.

How to interpret these seemingly conflicting findings? One possibility is that dTRPM could regulate both Mg²⁺ and Zn²⁺ homeostasis; alternatively, it could regulate one cation's homeostasis, which indirectly affects the other's; variations of metal levels in different genetic backgrounds could also contribute to some of the observations made. In terms of genetic background and zinc levels, it is worthwhile to note that Fanis Missirlis's group reported a recessive X-linked mutation which could cause a threefold reduction of body zinc in *Drosophila*³⁹. The identity of this gene, however, still remains a mystery.

4. Zinc absorption in *Drosophila*

Research into dietary zinc absorption in *Drosophila* started just a few years ago. Nevertheless, thanks to the genetic amiability of this powerful model organism, a relative comprehensive picture of zinc absorption in *Drosophila* has begun to emerge^{14,15,33,40}.

4.1. The digestive system of *Drosophila*

As shown in figure 1, in *Drosophila* food firstly passes through the foregut, proventriculus and then gets into the midgut and later the hindgut. Similar to mammalian intestine and stomach, *Drosophila* midgut is responsible for food digestion and absorption⁴¹. The middle part of the midgut or the middle midgut consists of copper cell, large flat cell and the iron cell region. The food uptaken is

acidified in the copper cell and goes ahead to past the large flat cell to be neutralized in the iron cell region, before getting into the posterior midgut. The majority of nutrient absorption is thought to occur in the posterior midgut⁴¹. Like mammalian large intestines, *Drosophila* hindguts function in water and ion reabsorption before faecal excretion. The malpighian tubules are the osmoregulatory and detoxifying organs of *Drosophila*; they are located at the boundary between the midgut and the hindgut, functioning together with the hindgut to regulate fluid and ion balance similar to mammalian kidneys⁴²⁻⁴⁵.

4.2. Dietary zinc influx on the apical membrane

Zinc absorption is performed by zinc uptake from the food at the luminal side through the apical membrane of the gut, the intracellular trafficking of zinc from the apical membrane to the basolateral membrane of the enterocyte, and zinc efflux into circulation for systemic use.

It is reported that mutations in ZIP1, ZIP2, or ZIP3 confer on mice decreased ability to survive under dietary zinc limitation, especially during pregnancy, when zinc requirement is increased⁴⁶⁻⁴⁸. However, direct supporting evidence for the involvement of ZIP1, ZIP2, and ZIP3 in mammalian dietary zinc absorption is still lacking. It is generally accepted that in human and mice the major dietary zinc uptake gene is ZIP4⁴⁹⁻⁵¹, while the relative importance of other ZIPs in dietary zinc absorption are less certain³.

We recently identified in *Drosophila* two functionally overlapping zinc importers of the ZIP family, which reside on the apical membrane of the enterocyte and are required for efficient dietary zinc absorption. These two ZIP proteins, CG9428 and CG9430, are involved in absorbing zinc from the lumen into the enterocyte¹⁴(figure 2A).

dZIP1, encoded by CG9428, is the closest homolog of hZIP1 in the fly genome and expresses intensively in the midgut constriction; dZIP2, encoded by CG9430, is a protein very closely related to CG9428 and immediately adjacent to CG9428 in the genomic DNA. Either ubiquitously or gut-specifically knocking CG9430 down results in sensitivity to zinc deficiency, whereas uniformly knocking CG9428 down did not cause significant defects either on normal food or on zinc-depleted food. A double RNAi of CG9428 and CG9430 results in more severe phenotypes than CG9430 knockdown alone; zinc supplementation in food was able to restore these phenotypes. These results suggest that both of them are zinc importers important for dietary zinc absorption. Though partially redundant, it appears that CG9430 is slightly more important than CG9428 or is a more potent zinc transporter when zinc is severely depleted¹⁴. Knocking down both of them lead to additional sensitivity to zinc deprivation than knocking down either one of them alone, but has minimal effect under normal dietary conditions, suggesting that there might be additional pathways for zinc absorption.

Richard Burke's lab also identified another ZIP family member CG6898, working in concert with CG9428 and CG9430 to ensure optimal zinc absorption (figure 2A). They provided evidences that CG6898 may be acting as a constitutive, low-affinity

zinc uptake transporter active in the posterior midgut that works together with CG9428 and CG9430 to ensure adequate zinc supply under a range of dietary conditions³³. Besides these relatively well characterized zinc uptake transporters, some other zinc transporters such as CG10006, may also participate in zinc absorption, but further studies are needed to validate this⁴⁰.

Among all the *Drosophila* ZIP genes expressed in the gut, only CG9428 and CG9430 were transcriptionally regulated by dietary zinc levels, with their RNA levels negatively correlated to zinc levels. It is noteworthy that zinc transporters may also subject to post-transcriptional controls, a question little addressed. For example, post-transcriptional regulation of CG9428 was detected at the midgut constriction when dietary zinc was limited¹⁴, and CG6898 could not be forcibly highly expressed in the gut while it was possible to do so in some other tissues³³.

4.3. Zinc sequestration and distribution

The intracellular zinc trafficking routes from the apical membrane to the basolateral membrane of the enterocytes remain poorly characterized. Zinc transporters residing on the membrane of intracellular organelles may act to sequester cytoplasmic zinc into various compartments for secretion, storage or for proteins that require zinc for their activities. In *Drosophila*, there are several possible intracellular ZnTs namely CG6672, CG31860, CG11163 and CG8632, and two ZIPs CG94283 and CG10449, as suggested by homology comparison analysis with mammalian zinc transporters (table 1). When they were RNA-interfered in the gut, no significant sensitivity to zinc was observed, suggesting they are not significant in dietary zinc absorption^{14 40}.

Some of these transporters deserve a little bit more description here. CG31860 is closer to a set of intracellular zinc transporters including ZnT2, 3 and 8. These transporters such as hZnT4 are found to be localized to the endosomal compartment or trans-Golgi network, and may mediate zinc secretion into the blood stream via exocytosis (reviewed in³). One homolog in *Caenorhabditis elegans*, CDF-2, is functional in zinc storage in the gut granules. CG31860 plays an important role in intracellular zinc distribution^{14,52,53}. But the vesicle-resident protein encoded by CG31860 plays an insignificant role in the process of dietary zinc absorption, as suggested by the lack of obvious phenotypes when its expression was knocked down^{14,52,53}.

CG6672 is the only one Golgi-resident ZnT family member. ZnT7-null mice displayed reduced body zinc levels that cannot be rescued by intravascular zinc injection⁵⁴. Based on studies in *Drosophila*, CG6672 is responsible for zinc efflux from the cytoplasm into the Golgi, but does not participate in dietary zinc absorption^{14,53}, suggesting the whole secretory pathway may not be involved in zinc efflux to the circulation.

The *Drosophila* genome harbors four metallothionein genes-*MtnA*, *MtnB*, *MtnC*, and *MtnD*. Metallothioneins play an important role in metal detoxification: *Drosophila* with all four *Mtns* knockout are sensitive to copper, cadmium and zinc load⁵⁵. These metals also induce *Mtns* expression in various regions of the gut. Though it is undisputable that *Mtns* play an important role in metal homeostasis, their

involvement in zinc or metal trafficking needs further elucidation and it remains to be seen whether their effects are direct or not.

4.4. Dietary zinc efflux at the basolateral membrane

In *Drosophila* zinc efflux from the enterocytes to the circulation is mediated by a collaborative function of CG17723¹⁵ and its close homolog CG5130¹⁴ (figure 2A).

CG17723 shares the highest homology with human ZnT1; it is the first zinc transporter identified for zinc absorption in *Drosophila*. Ubiquitous or gut-specific CG17723 knockdown leads to growth retardation and developmental arrest under zinc deficiency, which could be complemented by human ZnT1¹⁵. CG17723 resides on the basolateral membrane of the enterocytes in the midgut constriction and is responsible for zinc efflux for systemic supply¹⁵. CG17723 is strongly regulated in the gut post-transcriptionally¹⁴.

Another basolateral membrane localized zinc transporter, CG5130, shares with CG17723 26% identity and 48% similarity, and also participates in the exit of zinc from enterocytes to circulation¹⁴. Knocking down both of CG17723 and CG5130 resulted greater sensitivity to zinc deficiency as compared with knocking down either one of them alone¹⁴.

5. Zinc homeostasis in the malpighian tubules

Besides zinc absorption, zinc excretion is an additional way to control body zinc homeostasis. As shown in figure 1, *Drosophila melanogaster* has two pairs of malpighian tubules, functionally similar to kidneys in mammals. Malpighian tubules originate from the junction between the midgut and hindgut, with one pair migrating anteriorly and the other pair posteriorly^{45,56,57}. They work together to participate in osmoregulatory regulation and detoxification in *Drosophila*^{45,57-59}.

CG3994 is the only presently known member playing a role in zinc excretion in *Drosophila* (figure 2B). It was reported to localize to the plasma membrane both under normal and elevated zinc concentrations in malpighian tubules. When CG3994 is repressed or overexpressed, the body zinc level is correspondingly increased or decreased; CG3994 mutant flies are sensitive to zinc supplement and overexpression flies are resistant to high zinc levels. Expression of CG3994 is induced when zinc is supplemented in the food^{14,16}. CG3994 has putative transcription factor MTF-1 binding sites (or metal regulatory elements, MREs), and the transcript of CG3994 was markedly increased when MTF-1 was overexpressed, suggesting that the metal regulation of CG3994 in *Drosophila* is mediated by MTF-1¹⁶.

Besides the specific expression of CG17723 in the larval midgut constriction for dietary zinc absorption, CG17723 was also found in the malpighian tubules¹⁵ (figure 2B). Given the basolateral membrane localization of CG17723 in the malpighian tubules, we conjectured that it might be related to renal reabsorption of zinc there. Interestingly, a similar expression orientation of ZnT1 was also found in rodent kidneys⁶⁰.

These two zinc transporters are both zinc exporters. Theoretically a *Drosophila* zinc importer, which resides on the basal membrane of the malpighian tubules and is

required for zinc uptake into the malpighian tubules for excretion should exist (figure 2B). Identification of this transporter therefore would fulfill the missing link, i.e., zinc uptake from the body fluid in the malpighian tubule cells.

Interestingly, zinc levels in the malpighian tubules are related to a process of mineralization that could eventually lead to the stone formation. As shown in table 1, three ZnT transporters (CG3994, CG11163, and CG17723) are highly expressed in the malpighian tubules, implying a possible role in regulating tubule zinc homeostasis. Ubiquitously knocking-down these genes significantly reduced kidney stone formation in a fly calcification model generated by inhibiting xanthine dehydrogenase (Xdh)⁶¹. Dietary and pharmacologic modulation of zinc levels also altered *Drosophila* stone formation⁶¹. This suggests that these three transporters are directly or indirectly connected to the tubule zinc homeostasis, and zinc levels in the malpighian tubules are important to this physiologic process.

6. Zinc and neurodegenerations

Zinc dyshomeostasis may be connected to many diseases. Accumulation of metals, including zinc, is often found in pathologically affected regions of many neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington disease (HD) and so on^{62,63}. However, to a large extent, roles of metal ions in these diseases are not well established.

Drosophila has a sophisticated nervous system responsible for behaviors from simple avoidance to learning and memory; its compound eyes allow convenient phenotypic characterization. Many neurodegenerative diseases have been successfully modeled in *Drosophila*. Expression of human A β 42 in *Drosophila* eyes results in a degeneration of eye structures progressing with age⁶⁴. Walter Schaffner's lab found

dietary supplementation of zinc or copper ions could exacerbate the eye damage ; consistently, zinc/copper chelators, or over-expression of metal-responsive transcription factor 1 (MTF-1, a zinc-finger protein, a transcription factor with a key role in metal homeostasis and detoxification) or with human or *Drosophila* metallothionein transgenes alleviated the phenotype⁶⁴. Expression of human A β 42 in *Drosophila* brains recapitulates some major AD-like symptoms^{65,66}. Using this model, we found that genetic manipulation of the expression of certain zinc importers altered an array of phenotypes associated with A β 42 expression, including brain vacuolization, locomotor defects and reduced lifespan. Furthermore, we showed that zinc accumulation promotes both the SDS-soluble form and fibril deposits of A β 42 formation in *Drosophila* brains⁶⁷.

Mutations in the *Drosophila* ortholog of human Parkin lead to a series of defects mimicking human Parkinson's disease. MTF-1 mutation enhanced the phenotypes caused by Parkin loss-of-function; elevated expression of *Drosophila* or human MTF-1 or metallothionein improved the condition of Parkin mutants⁶⁸.

Insoluble aggregates of the microtubule-associated protein Tau are a common feature of several types of neurodegenerative diseases (including AD) collectively termed “tauopathy”. Expression of wild-type or mutant human Tau in the *Drosophila*

nervous system caused degenerations^{69,70}. In a *Drosophila* tauopathy model built upon the heterologous expression of hTauR406W (a mutant Tau found in some FTDP-17 patients), we found that zinc reduction and increase, either through genetic or dietary modulation, could respectively alleviate and exacerbate the Tau toxicities. Zinc's involvement in Tau toxicities mainly arises from its direct binding to Tau protein; mutation of the zinc binding sites in Tau greatly suppresses Tau toxicity and astonishingly, the toxicity is no longer affected by zinc level modulations. These studies provided new insights into zinc's exact roles in relevant neurodegenerative diseases, and could even have therapeutic implications.

7. Conclusive remarks

In the *Drosophila* proteome, seven members of the ZnT family and ten numbers of the ZIP family display homology to mammalian zinc transporters localized to the plasma membrane or intracellular compartments. Our understanding of *Drosophila* zinc metabolism suggests zinc homeostasis is a conserved evolutionary process; cellular zinc uptake, sequestration, distribution and efflux mechanisms appear rather similar, but not identical, between *Drosophila* and mammals. *Drosophila melanogaster* could serve as a tractable model to study the function of zinc transporters, and how an array of zinc transporters function in a coordinative manner during different biological processes. The use of *Drosophila* indeed advanced our understanding of systemic zinc homeostasis and its relevance to human diseases. It is expected that future studies in *Drosophila* will continue making contributions to our further understanding of zinc homeostasis overall.

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Figure legends

Figure 1 The *Drosophila* larval digestive system. Notable parts are the foregut, proventriculus, gastric caecae, midgut, malpighian tubules and hindgut. The red-boxed regions are the two domains where obvious zinc accumulation is observed. Using synchrotron X-ray fluorescence microscope (XFM), zinc was observed throughout the gastrointestinal tract with an increased accumulation in the crop region of the anterior midgut and the posterior midgut region⁷¹. Interestingly, elevated levels of dietary zinc lead to a marked increase in ferritin and metallothioneins protein levels in the posterior midgut^{15,72}, suggesting a role for the posterior intestine in zinc homeostasis.

Figure 2 Zinc absorption and excretion in *Drosophila*. (A) A model for dietary zinc absorption in *Drosophila* enterocytes. Dietary zinc absorption in the enterocytes likely occurs by directly transversing through the cell, and bypassing the intracellular organelles. (B) Zinc homeostasis in the malpighian tubules. For homologs and alternative names of these transporters please see table 1. For example, CG17723 is alternatively named dZnT1 or ZnT63C in different literatures.

Figure 1

Figure 2

(A)

(B)

Table1 Drosophila zinc transporters

Family	Symbol	Human homologs ⁵²	Subcellular localization	Expression pattern	Function in Drosophila	Phenotypes of mutation or ubiquitous RNAi
SLC30A(ZnT)	CG17723 dZnT1 dZnT63C	ZnT1 ZnT10	plasma membrane ¹⁵	prominent in the midgut constriction, malpighian tubules and also found in testis and salivary glands ¹⁵	exit of gut zinc to the circulation ¹⁵ ; facilitating zinc tubule excretion ⁶¹	sensitive on zinc depletion food ¹⁵
	CG5130 dZnT77C	ZnT1 ZnT10	both on the plasma membrane and intracellularly ¹⁸	present in central nervous (CNS) and tracheal of the embryo (BDGP), trachea, hindgut, tubule, midgut and CNS of larvae and male accessory glands, tubule of the adults (FlyAtlas)	exit of gut zinc to the circulation ¹⁴	sensitive on zinc depletion food ¹⁴
	CG31860 dZnT33D	ZnT2 ZnT3 ZnT8	both on the plasma membrane and intracellularly ¹⁸	abundant in testis, also some in larvae fat body (FlyAtlas)	unknown	lethal at embryo or 1 st larvae stage, no response to zinc (unpublished results)
	CG3994 dZnT35C	ZnT2 ZnT3 ZnT8	plasma membrane ¹⁶	prominent in malpighian tubules from embryo to adults (BDGP, FlyAtlas)	zinc excretion in malpighian tubules ^{16 61}	sensitivity to zinc supplemental in food ¹⁶
	CG11163 dZnT41F	ZnT4	solely intracellularly ¹⁸	present in brain, hindgut and midgut of the embryo (BDGP), head, midgut, hindgut , tubule, heart, fat body from larval to adults (FlyAtlas)	facilitating zinc tubule excretion ⁶¹	normal, no response to zinc (unpublished results)

	CG6672 dZnT7 dZnT86D	ZnT7	both on the plasma membrane and intracellularly ¹⁸ , mostly like Golgi ¹⁴	prominent in larval salivary gland, midgut, tubule, hindgut, fat body, trachea, carcass; salivary gland, midgut, hindgut, fat body and ovary of adults (FlyAtlas)	zinc efflux to the Golgi ¹⁴	lethal at 3 rd larvae stage, no response to zinc ¹⁴
	CG8632 dZnT49B	ZnT9	unknown	present in midgut and gonad of embryo (BDGP), abundant in adults brain, head, eye, crop, tubule and ovary (FlyAtlas)	unknown	lethal at late pupae stage, no response to zinc (unpublished results)
SLC39A(ZIP)	CG9428 dZIP1 dZip42C.1	ZIP1 ZIP2 ZIP3	plasma membrane ¹⁴	mainly expressed in the midgut, and is also present in trachea and testis (FlyAtlas)	absorbing zinc from the lumen into the enterocyte ¹⁴	normal, no response to zinc, enhance dZIP2's phenotype ¹⁴
	CG9430 dZIP2 dZip42C.2	ZIP1 ZIP2 ZIP3	both on the plasma membrane and intracellularly ^{14 18}	mainly expressed in the midgut, and is also present in eye, tubule and fat body (FlyAtlas)	absorbing zinc from the lumen into the enterocyte ¹⁴	sensitive on zinc depletion food ¹⁴
	CG6898 dZip89B	ZIP1 ZIP2 ZIP3	plasma membrane ³³	mainly expressed in hindgut, and is also present in head, midgut and tubule (FlyAtlas)	dietary zinc uptake ³³	resistant to high zinc level in food ³³
	CG4334 dZip88E	ZIP1 ZIP2 ZIP3	both on the plasma membrane and intracellularly ¹⁸	highly expressed in larval salivary gland, also present in tubule and ovary (FlyAtlas)	unknown	lethal at late pupae stage or early adult stage, could be rescued by zinc depletion in food (unpublished results)
	CG10006 dZip71B	ZIP5	unknown	upregulated in tubule, also present in brain, salivary gland and hind gut (FlyAtlas)	unknown	normal, no response to zinc (unpublished results)

	CG6817 foi	ZIP6 ZIP10	plasma membrane ²⁵	strongly expressed in adults fat body ovary, and larvae CNS (FlyAtlas)	required in the mesoderm for gonad morphogenesis and tracheal branch fusion during tracheal development ²⁵	lethal at 3 rd instar larvae or early pupae stage, no response to zinc (unpublished results)
	CG10449 catsup	ZIP7	ER, Golgi ³²	ubiquitous in embryo; expressed in salivary gland, tubule, trachea, hindgut, fat body and carcass in larval; eye, salivary gland, crop, midgut, tubule, hindgut, heart and fat body in adults (FlyAtlas)	negative regulator of tyrosine hydroxylase activity ²⁷	lethal at 1 st or 2 nd instar larvae, no response to zinc (unpublished results)
	CG7816 dZIP13 dZip99C	ZIP13	ER, Golgi ¹⁷	strongly expressed in midgut, tubule, heart and testis (FlyAtlas)	supplies iron into the secretory pathway ¹⁷	lethal at late pupae stage or early adult stage, no response to zinc, could be rescued by iron supplementation ¹⁷
	CG2177 dZip102B	ZIP9	unknown	strongly expressed in tubule, fat body, crop and thoracoabdominal ganglion (FlyAtlas)	unknown	
	CG13189 dZip48C	ZIP11	unknown	strongly expressed in tubule, hindgut, salivary gland and midgut (FlyAtlas)	unknown	