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A fly's eye view of zinc homeostasis: Novel insights into the genetic control of zinc metabolism from *Drosophila*☆

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ABSTRACT

The core zinc transport machinery is well conserved between invertebrates and mammals, with the vinegar fly *Drosophila melanogaster* having clear homologues of all major groups of mammalian *ZIP* and *ZNT* transport genes. Functional characterization of several of the fly genes has revealed functional conservation between related fly and mammalian zinc transporters in some but not all cases, indicating that *Drosophila* is a useful model for examining mammalian zinc metabolism. Furthermore, *Drosophila* research, sometimes quite serendipitously, has provided novel insights into the function of zinc transporters and into zinc-related pathologies, which are highlighted here. Finally, the future research potential of the fly in nutrient metabolism is explored, with reference to emerging experimental technologies.

1. Experimental advantages of *Drosophila*

Ever since Thomas Hunt Morgan used the *white* eye colour mutation to demonstrate X-linked inheritance in *Drosophila melanogaster* [1], the vinegar fly has played a central role in studying the genetic regulation of animal development, physiology and behaviour. With only four chromosomes and boasting a host of useful genetic tools such as visible dominant marker alleles, balancer chromosomes to suppress recombination and multiple techniques to generate loss- and gain-of-function mutations, *Drosophila* rapidly became the model organism of choice for many geneticists in the 20th Century.

With the advent of the molecular age, the genetic toolkit available to *Drosophila* researchers has expanded dramatically, providing unparalleled capacity to manipulate and monitor gene activity *in vivo*. Exhaustive reviews of current fly molecular genetic techniques are provided elsewhere [2–5]. For the purposes of this review, it suffices to highlight the most relevant experimental advantages.

First, the generation of any type of transgenic fly strain is now routine and many *Drosophila* laboratories outsource this work for a modest fee to commercial operators. The short generation time (typically 10 days from embryogenesis to adulthood), large progeny numbers and ease of maintenance mean that several dozen or even hundreds of transgenic strains can be generated and maintained by a small research group.

While the generation of gene knockout strains (where the endogenous gene has been deleted or inactivated by a null mutation) can be informative, functional characterization of such mutations can be hindered in situations where the gene is essential and the mutants therefore die early in embryonic or larval development. This problem can be circumvented in the fly either by: 1) generating mosaic animals with marked patches of homozygous mutant tissue in an otherwise heterozygous individual [6]; or 2) using the GAL4/UAS bipartite gene manipulation system, whereby gene over expression or RNA interference (RNAi) knockdown transgenic constructs are activated in an inducible or tissue-specific manner using driver lines that express the GAL4 transcription factor under the control of chosen enhancer sequences [5]. Hundreds of GAL4 strains are publically available, allowing exquisite control of individual gene activity and RNAi lines for almost every *Drosophila* gene can be purchased for a small fee. However, one

Abbreviations: ER, Endoplasmic Reticulum; TPEN, *N,N,N′,N′*-tetrakis(2-pyridylmethyl)ethane-1,2-diamine; XFM, x-ray fluorescence microscopy; RNAi, RNA interference; MTF-1, metal transcription factor-1; XD, xanthine dehydroxylase; ZFTF, zinc-finger transcription factor.

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major caveat with the use of RNAi is that gene knockdown is rarely 100% effective, meaning that arguments can often be made for residual gene activity.

2. Potential use of *Drosophila* in zinc biology

Compared to the extensive use of *Drosophila* to study neurobiology or processes such as tissue growth and patterning, the volume of research on nutrient metabolism in the fly is relatively modest. Nonetheless, the experimental advantages of invertebrates lend themselves to metabolic studies too. In particular, the ability to manipulate individual genes in specific cell types allows in-depth functional characterization of genes thought to be involved in nutrient uptake, distribution or excretion. Furthermore, the *Drosophila* genome often contains fewer genes of a particular family compared to mammalian genomes, reducing the potential for functional redundancy or overlap between closely-related genes. Dietary content and composition can be tightly controlled in the fly and physiological responses to changes in diet can be monitored *in vivo* in real time through the use of fluorescence-based reporter genes.

3. Conservation of the core zinc homeostasis machinery

To determine whether *Drosophila* may provide a relevant model for studying zinc homeostasis, we first must ask whether the core zinc transport and regulation machinery discovered in yeast and mammals is conserved in the fly. Fortunately for this review, the answer is a resounding 'yes'.

Transport of zinc across cellular membranes into the cytosol is chiefly facilitated by ZIP family proteins. The *Drosophila* genome encodes 10 such proteins compared to the 14 found in mouse and human genomes [7]. For the purposes of this review, the fly zinc transport genes will mostly be referred to by their chromosomal location; for instance, *ZIP71B* refers to the *ZIP* gene found at cytological position 71B on the third chromosome. This nomenclature, adopted from one of the earlier articles on fly zinc physiology [8], avoids the potential pitfalls of assigning functional homology between invertebrate and mammalian genes. In some cases, where a gene was identified by mutant phenotype before it was recognised as a zinc transporter, the historical gene name will be used (e.g. *FOI*, *CATSUP*). In instances where researchers have given alternative gene names based on demonstrated orthology between the mammalian and fly gene (e.g. *dZNT1*), these names will also be provided. A list of all *Drosophila ZIP* and *ZNT* genes and their most similar mammalian homologues is given in Table 1.

Phylogenetic analysis comparing the amino acid sequences of human and *Drosophila* ZIP proteins showed that homology could be found between each of the fly transporters and one or more mammalian ZIP proteins [7]. In many cases (e.g. ZIP84C, ZIP102B, ZIP99C/dZIP13, CATSUP, ZIP71B), there appears to be a single mammalian transporter most closely related to the corresponding fly transporter (Table 1). In contrast, mammalian ZIPs 1, 2 and 3 are clearly related to ZIP88E, ZIP89B, ZIP42C.1 (dZIP1) and ZIP42C.2 (dZIP2) but assigning individual relationships between these seven proteins would be risky. Similarly, ZIPs 6 and 10 appear most closely related to FOI yet ZIPs 4, 8, 12 and 14 have no obvious fly counterpart.

The ZNT zinc efflux proteins show a similar relationship pattern to that seen in the ZIPs [7]. One-to-one homology is seen between ZNT9 and ZNT49B. ZNT86D (dZNT7) appears to be the only fly homologue of the Golgi-localized ZNTs 5, 6 and 7. ZNTs 1 and 10 cluster together with ZNT63C (dZNT1) and ZNT77C while ZNTs 2, 3, 4 and 8 fall into a larger clade with the fly ZNTs 41F, 35C and 33D.

It should be noted that direct demonstration of *in vivo* zinc transport activity has not been achieved for most of the fly ZIP and ZNT proteins; exceptions will be discussed in the course of this review. Such a demonstration typically involves examining the kinetics of ^{65}Zn accumulation in cultured cells or complementation of zinc transport defects in mutant yeast strains, experimental approaches to which the fly is not amenable. In the absence of direct zinc transport assays, *Drosophila* researchers typically rely on indirect measures such as the expression of zinc-responsive genes, the activity of zinc-dependent enzymes or the tolerance of mutant flies to alterations in dietary zinc levels to demonstrate probable zinc transport activity.

Moving beyond the zinc transporters, the other key components of cellular zinc regulation in mammals are metal transcription factor-1 (MTF-1) and its transcriptional targets, the *Metallothionein* genes. *Drosophila* also has a single *MTF-1* gene and five *metallothionein* genes (A to E) reviewed in Ref. [9]. Due to space constraints this important group of genes will not be discussed in detail in this review.

Table 1
List of *Drosophila ZIP* and *ZNT* genes (including synonyms) and their closest mammalian homologues. *While most of the mammalian ZIP and ZNT genes have been directly demonstrated to transport zinc, ZNT10 has been shown to have greater transport activity for manganese. **Similarly, there is considerable evidence that the *Drosophila* ZIP13 homologue is actually an iron transporter.

Drosophila ZNT and *ZIP* genes	Closest mammalian homologues
ZNT41F, CG11163	ZNTs 2, 3, 4 and 8
dZNT1, ZNT63C, CG17723	ZNTs 1 and 10*
ZNT33D, CG31860	ZNTs 2, 3, 4 and 8
ZNT35C, CG3994	ZNTs 2, 3, 4 and 8
ZNT77C, CG5130	ZNTs 1 and 10*
ZNT86D, dZNT7, CG6672	ZNTs 5, 6 and 7
ZNT49B, CG8632	ZNT9
ZIP88E, CG4334	ZIPs 1, 2 and 3
ZIP42C.2, dZIP2, CG9430	ZIPs 1, 2 and 3
ZIP42C.1, dZIP1, CG9428	ZIPs 1, 2 and 3
ZIP89B, CG6898	ZIPs 1, 2 and 3
fear of intimacy (FOI), CG6817	ZIPs 6 and 10
ZIP99C**, dZIP13**, CG7816**	ZIP 13
catecholamines up (CATSUP), CG10449	ZIP 7
ZIP71B, CG10006	ZIP 5
ZIP102B, CG2177	ZIP 9
ZIP48C, CG13189	ZIP 11

4. Localization and quantification of zinc in flies

One approach to gauge the importance of zinc in specific tissues or organelles is to determine whether zinc accumulates to a greater extent in certain cell types or subcellular compartments. Unfortunately, the small size of the fly can prove to be a hindrance for biochemical analyses; gaining enough material for reliable ion quantification by atomic absorption spectroscopy/inductively coupled plasma-mass spectroscopy for instance is difficult, particularly if different tissue types are to be analysed separately or if cellular fractionation is required. One way to circumvent this issue is through spatial mapping of ion content. Early attempts at this with microprobe analysis of the dissected gastrointestinal tract revealed particularly high levels of zinc in the Malpighian tubules [10] - interestingly the site of ectopic calcification in the *Drosophila* kidney stones model [11].

Recently the Burke laboratory has used x-ray fluorescence microscopy (XFM) to map the distribution of zinc, iron and copper in several fly tissues. In the eye and wing imaginal discs, zinc, like iron, appeared to be evenly distributed across these two-dimensional epithelia, in contrast to copper which accumulates at the margins of the wing disc [12]. The wing disc was also used to show that targeted manipulation of zinc transport (upregulation of *ZIP42C.1*

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