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A recessive X-linked mutation causes a threefold reduction of total body zinc accumulation in *Drosophila melanogaster* laboratory strains☆

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ABSTRACT

A newly identified human locus on chromosome 15 was recently associated with zinc accumulation. Based on a prior report of a threefold difference in zinc accumulation between *fumble*[1] heterozygous mutants and control fly strains, it was suggested that phosphopantothenoylcysteine decarboxylase might affect zinc status through its effects on vitamin B5 (pantothenate) metabolism. We report here that outcrossed *fumble*[1] heterozygous mutant flies with low zinc content have been recovered, suggesting that pantothenate metabolism did not alter zinc homeostasis in *fumble*[1] heterozygous flies. We show instead that the *Drosophila* condition of low body zinc accumulation is an X-chromosome-linked recessive trait.

1. Introduction

Zinc serves as a co-factor for many protein families with diverse functions; notable examples are hydrolases and zinc-finger transcription factors [1]. A role for zinc in enzyme catalysis was first shown for carbonic anhydrase [2,3] and in DNA binding for transcription factor IIIA of *Xenopus* [4–6]. Zinc accumulates in pancreatic granules [7–9] and mossy fibers of the hippocampus [10–12], where its function remains to be established [13–16]. Variations in intracellular concentrations of free zinc are thought to contribute to cell signaling [17,18]. Zinc deficiency is widespread in humans and can lead to growth retardation, hypogonadism in males, rough skin, impaired immunity and neurological defects [19]. Therefore, it was surprising to discover *Drosophila melanogaster* strains raised under the same (zinc replete) dietary conditions and bearing a threefold difference in their total body zinc content had no obvious phenotypic defects [20].

A few aspects of zinc homeostasis have been studied in *Drosophila*, including functional studies of zinc transporters [21–25], of the zinc-responsive metal transcription factor-1 (MTF1) [26–29] and of metallothioneins [30–33], which are under MTF1 control. In addition, the neurotoxic properties of zinc in neurodegenerative disease have been demonstrated in models of Alzheimer's disease [34,35], Parkinson's disease [36,37], as well as in activating apoptosis in *Drosophila* hemocytes [38]. Moreover, a study of the global transcriptional response to dietary zinc has been published [39].

In our studies of metal determinations on laboratory strains from diverse genetic backgrounds we consistently found total body zinc values below a threshold of 100 mg Zn/g dry body weight [40–42]. In contrast, two loss-of-function *fumble* (*fbl*) mutant strains (a P-element insertion and a deficiency) had zinc accumulated at 200 mg/g dry body weight [20]. As *fbl* encodes for pantothenate kinase [43], the first enzyme involved in the metabolism of Vitamin B5, which is a precursor of coenzyme A, our initial hypothesis was that intermediary metabolism might affect zinc homeostasis in some way. This idea was recently noted by investigators who performed a genome wide association study in humans for loci affecting, amongst other elements, zinc accumulation in the blood [44]. A gene encoding phosphopantothenoylcysteine decarboxylase was present at a chromosome 15 locus associated with changes in zinc accumulation [44]. The human study prompted us to expedite the present report, because we have in the meantime refuted our original hypothesis associating *fbl* heterozygosity with zinc accumulation. Indeed, when we measured metal composition of the different *Drosophila* species the values determined for zinc were on the range of 200 mg/g dry body weight [45], which we now show to be the range of zinc accumulation also for wild type *D. melanogaster*. We report that a recessive X-linked mutation causes a threefold reduction of total body zinc accumulation in many *D. melanogaster* laboratory strains. Our results are not only pertinent for the community of metal biologists that use *Drosophila*,

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but could be significant in experiments involving zinc transcription factors and other zinc-containing proteins in the fruit fly.

2. Materials and methods

2.1. Fly maintenance

D. melanogaster were reared at 25 °C on a standard diet containing: agar (6.5%), sucrose (9.7%), glucose (21.3%), yeast (22.6%), maize (9.7%), treacle (19.3%), soya flour (4.6%), propionic acid (0.5%) and nipagin (0.01%). The *fbl*^{1} stock was obtained from Bloomington Drosophila Stock Center at Indiana University (#11777). The wild type strain we used was collected by Rudi Costa from Tannes, Italy and termed *Tan3* [45]. Balancer strains and *white* mutants were from our core lab stocks.

2.2. Flame atomic absorption spectrometry

The metal concentration of zinc in flies was determined by flame atomic absorption spectrometry. With a single exception mentioned in the text, male and females flies were used in combination and 100 mg dry mass was typically collected for each biological replicate. For all experiments shown we used 5 biological replicates. 4- to 7-day old flies were collected, fast-frozen in liquid nitrogen and stored at −80 °C. Samples were freeze-dried for 24 h and their dry mass was measured. Dried flies (100 mg) were acid digested by adding 1.5 ml of 69% nitric acid (HNO3) at 50 °C for 4 h, then at 100 °C for another 4 h, followed by overnight cooling down. Acid-digested samples were diluted with distilled water and the metal content was determined by using an AAnalyst 200 Flame Atomic Absorption Spectrophotometer (Varian Ltd., Yarnton, Oxfordshire, UK). Standards of each metal were used to calibrate the spectrophotometer and calculate metal concentrations in all samples.

3. Results and discussion

In a survey of elemental composition of some of our stocks, we were surprised to discover that a rebalanced *fbl*^{1}*/TM6, Tb* stock – generated during our previous study of this mutant's survival rate to the pupal stage of development [20] – accumulated a low amount of zinc (Fig. 1A). This observation suggested that *fbl*^{1} was not involved in zinc accumulation. Low zinc was a feature of all other lab stocks we tested, but a wild type reference stock termed *Tan3* [45] accumulated threefold more zinc, similar to the original *fbl*^{1}*/TM3* (Fig. 1A). Zinc accumulation in wild type *Tan3* flies was consistent with values observed in many other *Drosophila* species [45]. Our first attempt to explain the new findings was to test for the presence of maternal factors that could influence metal homeostasis, such as the presence of *Wolbachia* endosymbionts [46–48]. However, crossing of low zinc female *fbl*^{1}*/TM6* to high/normal zinc male *fbl*^{1}*/TM3* flies and exchanging the balancers resulted in new stocks with high/normal zinc accumulation (data not shown). This result meant that (i) the trait of "low zinc accumulation" was not due to a maternal factor, as low zinc *fbl*^{1}*/TM6* females did not transmit it to their progeny, (ii) the 3rd chromosome was not determining zinc concentration in the flies, as the same combination of 3rd chromosomes could result in different zinc accumulations and (iii) the trait of low zinc was recessive, as it disappeared when the two phenotypes were crossed against each other; this was confirmed in the reverse cross of high/normal zinc female *fbl*^{1}*/TM3* to low zinc male *fbl*^{1}*/TM6* flies.

After excluding, by use of appropriate balancers, a role for the 2nd chromosome, we tested if a recessive mutation on the X-chromosome could be causing the low zinc phenotype. For this experiment, we separated the flies by sex prior to determining the metal concentration, because females would serve as heterozygous controls, whereas males would inherit the X-chromosome from their mothers. Indeed,

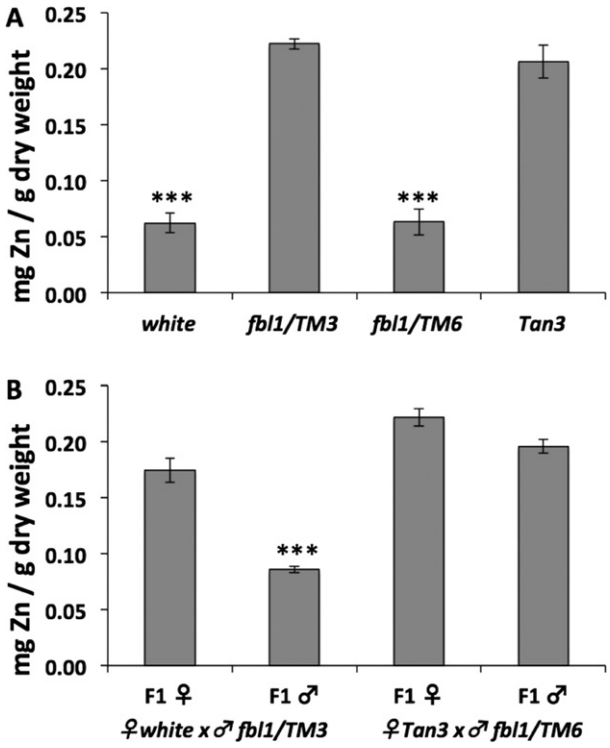

Fig. 1. Zinc content in different genotypes of *Drosophila melanogaster*. (A) Zinc content was measured by atomic absorption spectrometry. *white* and *fbl*^{1}, *ry*^{506}*/TM3, ry*^{RK}, *Sb, Ser* (abbreviated *fbl*^{1}*/TM3* throughout the text) were the same stocks as in [20], whereas metal content of *fbl*^{1}, *ry*^{506}*/TM6, Tb* (abbreviated *fbl*^{1}*/TM6, Tb* throughout the text) flies was assessed for the first time here. Note that *fbl*^{1} heterozygosity does not correlate with total body zinc accumulation. Analysis of Variance indicated significant differences between the genotypes with $p < 0.001$. (B) Zinc content measured in female and male progeny derived from indicated crosses. Note that male progeny derived from low zinc mothers is also low in zinc, suggesting that the maternal X-chromosome is responsible for the low zinc phenotype. Analysis of Variance indicated significant differences between samples with $p < 0.001$.

all male progeny collected from low zinc *white* mothers crossed to high/normal zinc *fbl*^{1}*/TM3* fathers were low in zinc (Fig. 1B). As expected from the recessive nature of the mutation we were following, all female progeny from the same cross were high/normal in zinc accumulation. Finally, when male progeny was derived from high/normal zinc *Tan3* females crossed to low zinc *fbl*^{1}*/TM6, Tb* males they were high/normal in zinc themselves (Fig. 1B). Therefore, we can conclude that a recessive X-linked mutation causes a threefold reduction of total body zinc accumulation in many *D. melanogaster* laboratory strains.

Although efforts in mapping the mutation are ongoing, we felt that raising awareness of the presence of this mutation was pertinent in order (i) to correct the assumption we previously published that pantothenate kinase was associated with zinc homeostasis [20], (ii) to avoid misleading efforts to map human genes involved in zinc homeostasis [44] (it is interesting to note that an X-chromosome linked locus affecting zinc homeostasis in humans was also detected in this study), (iii) to inform on the choice of control flies, as the previously unrecognized variation may have influenced other studies, including the suggestion that TRPM channels transport zinc [49,50] and (iv) to highlight the ability of fruit flies to survive in the lab with threefold less zinc, a finding that begs an answer on where the excess zinc may be stored and how zinc is distributed (preferentially or not) to target destinations in wild type flies.

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