

C. elegans as a model for Friedreich Ataxia

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In a recent FASEB paper, Palau and co-workers claimed that lowering frataxin reduces *C. elegans* lifespan (1). Given the importance of frataxin for survival in other organisms, these results may not seem surprising. However, they are clearly in contrast with our previously published findings showing that frataxin RNAi knock down, (both feeding and injection), increases *C. elegans* lifespan (2). Our results reinforce studies showing that inactivation or reduced expression of several different mitochondrial proteins increase lifespan (3, 4). While it is not uncommon for research groups to obtain contrasting results, what captured our attention in the Palau paper was that it misrepresented our experimental procedures and trivially dismissed the significance of our findings.


Both groups utilized RNAi to reduce frataxin expression. Palau and colleagues injected *fih-1* dsRNA directly into the gonads of fourth-larval stage (L4) larvae and followed their offspring's lifespan; we microinjected *fih-1* dsRNA into the gonads of 5-day-old adults and, similarly, examined the lifespan of the offspring of the injected animals. Palau and coworkers incorrectly state (1) that we utilized the injected adult animals for further study and consequently saw “no way to compare both kinds of experiments.” Any expert in nematode biology would immediately question such an experimental approach and consequently the validity of any results so obtained.

What then might explain the opposite effects on lifespan observed by the two groups following *fih-1* interference? We can envision at least two explanations for these contrasting results. The most immediate relates to the possibility that Palau's group analyzed their lifespan data differently. Specifically, under some environmental conditions, adult *C. elegans* will often retain their eggs internally resulting in a premature form of death (referred to as “bagging”). This is distinguishable from true age-related death and is generally censored in lifespan analyses. If bagged adults are not ascertained appropriately, mean lifespan will appear to be shortened. Given the egg-laying defective (Egl) phenotype reported by Palau et. al., (and shown in their Figure 2C), internal hatching of progeny was probably a frequent occurrence. It is not clear from their methods section if Palau et. al. censored bagged animals or not. To this end, however, and in agreement with our own results, it is informative that Palau et. al. reported

an increase in the lifespan of *fih-1* RNAi-treated animals when lifespan was carried out in the presence of FudR. This chemical sterilizes the animals thus preventing bagging. Given that the authors were aware of our publication, it seems unusual that this data was not shown and not considered further. A second explanation for the divergent results might come from variation in experimental conditions -use of different dsRNA constructs and/or microinjecting worms of different ages. For many genes RNAi efficacy can vary substantially depending on the specific dsRNA construct employed. In the study by Palau et al., dsRNA was generated from a fragment comprised of the entire *fih-1* ORF along with part of the 5'-UTR. In our studies two different dsRNA constructs were used—one based on the *fih-1* cDNA (for feeding experiments) and another based on the full-length genomic fragment (for injection studies). It is also well known that microinjection of dsRNA results in a more drastic reduction of gene expression than simply feeding animals dsRNA via their bacterial diet. In our own published studies on frataxin, we found that microinjection of *fih-1* dsRNA was more effective than feeding RNAi at causing a reduction in size of offspring (even when compared to animals whose ancestors had been continuously fed RNAi for 3 prior generations). Despite this, and in contrast to the findings of Palau and colleagues, we still observed that the progeny of microinjected animals lived longer, not shorter, than controls. Nevertheless, we suspect that frataxin RNAi knock down is more severe in the progeny of worms injected as L4 than in those injected at 5 days of age, since the latter are approaching the end of their fertile period and the gonad has already aged somewhat by this time. Intriguingly, this would imply that lowering frataxin below a critical threshold is not tolerable by *C. elegans* thus resulting in the shorter lifespans observed by Palau and colleagues.

In a manuscript currently under review, we show that longevity can be modulated by titrating mitochondrial gene expression. In those studies, we unambiguously demonstrate that life extension is observed only under reduced mitochondrial functionality and at greater reductions a shortening of lifespan is observed. Similar threshold effects are apparent in several human mito-

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chondrial-associated diseases. Notably in Friedreich Ataxia, where, the level of expression of frataxin must be below 75% normal in order for patients to present with a pathology; signs and symptoms directly correlate with protein expression level (5). In light of these observations showing mitochondrial threshold effects that dictate lifespan outcome, our experiments and those of Palau and colleagues may not be in conflict and together may in fact provide a better comprehension of both Friedreich Ataxia pathogenesis and mitochondrial control of lifespan. 

REFERENCES

1. Vazquez-Manrique, R. P., Gonzalez-Cabo, P., Ros, S., Aziz, H., Baylis, H. A., and Palau, F. (2005) Reduction of *Caenorhabditis*

- elegans* frataxin increases sensitivity to oxidative stress, reduces lifespan, and causes lethality in a mitochondrial complex II mutant. *FASEB J.* doi: 10.1096/fj.05-4212fje
2. Ventura, N., Rea, S., Henderson, S. T., Condo, I., Johnson, T. E., and Testi, R. (2005) Reduced expression of frataxin extends the lifespan of *Caenorhabditis elegans*. *Aging Cell* **4**, 109–112
3. Dillin, A., Hsu, A. L., Arantes-Oliveira, N., Lehrer-Graiwer, J., Hsin, H., Fraser, A. G., Kamath, R. S., Ahringer, J., and Kenyon, C. (2002) Rates of behavior and aging specified by mitochondrial function during development. *Science* **298**, 2398–2401
4. Lee, S. S., Lee, R. Y., Fraser, A. G., Kamath, R. S., Ahringer, J., and Ruvkun, G. (2003) A systematic RNAi screen identifies a critical role for mitochondria in *C. elegans* longevity. *Nat. Genet.* **33**, 40–48
5. Patel, P. I., and Isaya, G. (2001) Friedreich ataxia: from GAA triplet-repeat expansion to frataxin deficiency. *Am. J. Hum. Genet.* **69**, 15–24

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