RESEARCH COMMUNICATION

Starvation and oxidative stress resistance in Drosophila are mediated through the eIF4E-binding protein, d4E-BP

Gritta Tettweiler,1,2 Mathieu Miron,2,3 Mark Jenkins,1 Nahum Sonenberg,2 and Paul F. Lasko1,4

1Department of Biology, McGill University, Montréal, Québec H3A 1B1, Canada; 2Department of Biochemistry and McGill Cancer Center, McGill University, Montréal, Québec H3G 1Y6, Canada

Life span of females was longer than for males (Fig. 1B), but a comparable relative effect of the null mutation in d4E-BP gene and the activity of d4E-BP protein.

Here we investigated whether d4E-BP is essential under starvation and oxidative stress conditions, because dFOXO activates the transcription of d4E-BP and d4E-BP mRNA levels increase upon starvation (Zinke et al. 2002). We also provide evidence that d4E-BP activity is linked to life span, as overexpression of dFOXO is linked to increased longevity (Giannakou et al. 2004; Hwangbo et al. 2002). We also provide evidence that d4E-BP is the critical effector of the dFOXO-induced stress-sensitive phenotype.

Results and Discussion

To investigate whether 4E-BP activity influences life span in Drosophila melanogaster, we determined the median life span of d4E-BPnull flies in comparison to revertant flies that were produced by a precise excision of the P[lacW] insertion of Thor (which disrupts the gene encoding d4E-BP) but which otherwise have the identical genetic background (Rodriguez et al. 1996; Bernal and Kimbrell 2000; Bernal et al. 2004; and see Supplementary Fig. S1 for details of the strain construction). These will subsequently be referred to as revertant flies. A null mutation in d4E-BP caused a significant decrease in longevity [Fig. 1]. The median life span of mutant males was 19.8 d, ~25% shorter than that of control males [Fig. 1A, median life span of 26.6 d]. The life span of females was longer than for males (Fig. 1B), but a comparable relative effect of the null mutation in d4E-BP on life span was observed in both sexes. These results show that d4E-BP, a target of the conserved PI3K/TOR signaling pathway that has been strongly implicated in longevity, has a significant impact itself on life span.

In Drosophila larvae, protein-rich nutrition is critical for survival, while adults can survive up to 3 wk without protein. Strikingly, we observed that larval d4E-BP protein levels rise rapidly during nutritional stress [Fig. 2]. A dramatic increase of ~10-fold is observed after 8 h of starvation. To determine whether this increase in d4E-BP level is important for survival following starvation, we collected eggs from Oregon-R, d4E-BPnull, and revertant flies, transferred them to starvation medium, and scored mortality at 12-h intervals. Drosophila 4E-BPnull larvae die substantially faster than their control counterparts under starvation [Fig. 3, median life span of 20.8 h for starved d4E-BPnull larvae, 26.4 h for revertant, and 26.2 h for Oregon-R larvae]. To examine whether this effect can be overcome by induced expression of a d4E-BP(wt) transgene in a d4E-BPnull background, we used the UAS-GAL4 system (Brand and Perrimon 1993) with

[Keywords: Stress response, D. melanogaster, d4E-BP, dFOXO; translational control]

A rapid response is a crucial early line of defense in preventing cellular death in situations of stress (Sonenberg et al. 2000). Translational regulation allows an organism to generate quick responses to environmental cues by controlling the expression of protein from existing cellular mRNAs (Johnstone and Lasko 2001). Translation initiation of most eukaryotic mRNAs requires binding of eIF4E, the mRNA 5’ cap-binding protein, to the 5’ cap structure (Gingras et al. 1999). eIF4E, the cap-binding protein, is regulated by its cap-binding protein, is regulated by its
Role of d4E-BP in metabolic stress response

Figure 1. Life span of D. melanogaster is affected by the elf4E inhibitory protein, 4E-BP. (A) The median life span of d4E-BPnull males (n = 97) is ~25% reduced compared with revertant males (n = 157). (—) d4E-BPnull flies. (△) revertant control. Statistical comparison (log-rank test): revertant control vs. d4E-BPnull, p < 0.0001. (B) A null mutation in d4E-BP has a comparable effect on the life span of female animals. Statistical comparison (log-rank test): revertant control (n = 270) vs. d4E-BPnull (n = 165), p < 0.0001.

Figure 2. Time course of increasing expression of d4E-BP in response to nutrient stress: 40-h-old larvae of revertant animals were subjected to complete starvation. Identical amounts of total protein (30 µg) were analyzed by Western blot with 1868 antibody to d4E-BP. Loading control, α-tubulin.

Figure 3. d4E-BP binding to elf4E is required to rescue nutritional stress resistance. Eggs were subjected to complete starvation, and surviving larvae were counted every 12 h. d4E-BPnull larvae (∆—∆) died significantly faster than revertant control larvae (∆—●). This effect can be rescued by overexpression of d4E-BP (—●—●), but not by overexpression of d4E-BP[Y54A,M59A] (—□—□), a mutant form that cannot bind to elf4E. Statistical comparison (log-rank test): revertant control vs. d4E-BPnull, Hsp70-GAL4/UAS-d4E-BP(Y54A,M59A) (—□—□), a mutant form that cannot bind to elf4E, and d4E-BPnull flies (Fig. 4, median life spans of 34.6 h and 23.2 h, respectively). The survival rate at 60 h after exposure to oxidative stress was 0% for d4E-BPnull animals, and only 2% for dfoxo-null flies, compared with 66.1% for wild-type controls. Thus, an intriguing possibility is that d4E-BP acts as the downstream mediator of the dfoxo-null phenotype. As is the case for d4E-BP-mediated protection against starvation, this effect can be rescued by ectopic expression of d4E-BP [Fig. 4, median life span of 55.4 h]. The resistance to oxidative stress was also dependent on elf4E binding, since d4E-BP[Y54A, M59A] was unable to rescue stress sensitivity [Fig. 4, median life span of 24.1 h, 0.4% survival rate at 60 h]. The heat shock itself did not significantly affect life span (see Supplementary Fig. S3). To examine whether reduced levels of d4E-BP protein could account for the oxidative stress sensitivity of dfoxo-null mutants, we ectopically expressed d4E-BP in a dfoxo-independent manner in these animals. Remarkably, ectopic expression of d4E-BP completely rescued the sensitivity of dfoxo-null animals to oxidative stress [Fig. 4, median life span of 55.4 h]. Taken together, these results demonstrate that d4E-BP is a critical downstream mediator of dfoxo in oxidative stress resistance.

Normal growth and development are suspended during stress in order to concentrate resources on an appropriate stress response, and repressing translation does, in fact, slow growth [Groll et al. 2002; Patel et al. 2002]. We have shown that d4E-BP is strongly up-regulated during starvation, and its absence leads to compromised sur-
vival under outright starvation. The importance of d4E-BP in nutrient stress response is underscored by the fact that it is one of a small set of 14 genes whose expression is up-regulated as a consequence of starvation [Zinke et al. 2002, this study]. Our data support the current models to explain how oxidative stress activates transcription of d4E-BP. Oxidative stress promotes dephosphorylation of dFOXO, which causes its transport into the nucleus and activation of d4E-BP transcription [Jünger et al. 2003]. Amino acid starvation activates the dTOR pathway in the larval fat body. This triggers a starvation signal that suppresses the Inr/PI3K pathway in peripheral tissues [Colombani et al. 2003]. Suppression of this pathway results in inactivation of dAkt, which in turn can no longer phosphorylate and inactivate dFOXO, causing enhanced transcription of d4E-BP [Fig. 5].

Since the function of d4E-BP in stress resistance requires its eIF4E-binding activity, we propose that it involves repression of cap-dependent translation, concomitant with the stimulation of cap-independent translation. The up-regulation of d4E-BP could result in preferential translation of mRNAs, which translate in a cap-independent manner, via an internal ribosome entry site (IRES) element. Such stimulation has been documented under stress conditions including irradiation, hypoxia, and nutrient deprivation [Holcik et al. 2000]. It is also possible that d4E-BP exerts its effects by inhibiting the translation of key mRNA targets as was documented in mammals [Gingras et al. 1999]. Mammalian eIF4E preferentially stimulates the translation of mRNAs with a high degree of secondary structure in their mRNA 5′UTRs [Gingras et al. 1999]. These mRNAs, which are inefficiently translated, mostly encode proteins that play important roles in cell growth and proliferation [van der Velden and Thomas 1999].

Recent studies show that overexpression of dFOXO in adult fat bodies can extend the Drosophila life span [Giannakou et al. 2004; Hwangbo et al. 2004]. Further, overexpression of dtsc1, dtsc2, or dominant-negative forms of dTOR or ds6k all extend the life span of Drosophila [Kapahi et al. 2004]. Interestingly, TOR also regulates life span in Caenorhabditis elegans [Jia et al. 2004]. Our data support a role for d4E-BP in mediating life span, most likely as an effector of dFOXO. Taken together, our results add to the increasing body of evidence supporting a key role for eIF4E-mediated regulation of eIF4E in controlling cell growth, proliferation, and survival [Miron et al. 2001; Fingar et al. 2002; Li et al. 2002; Avdulov et al. 2004; Bjornstil and Houghton 2004].

Materials and methods
Drosophila strains

d4E-BPnull and revertant control flies are as described in Supplementary Figure S1. dFOXO-null alleles dFOXO21 and dFOXO25 are as described [Jünger et al. 2003].

Life span assay

For life span assays, male and female flies from a 24-h eclosion period were collected separately, and maintained at 25°C on standard cornmeal/agar medium in cages. Every 3 d, flies were transferred to fresh medium.
and the survivorship was determined. For analysis, the median life span was calculated as 50% survival.

**Stress treatments**

Eggs were collected from females for 2 h, separated from media, rinsed with 70% ethanol for 10 sec, rinsed with distilled H2O2, and placed individually into 200-µL PCR tubes (Abgene). Tubes contained 100 µL of starvation media, with single air holes poked in the lids. Survivors were individually into 200-µL PCR tubes (Abgene). Tubes contained 100 µL of antibody was performed with peroxidase-coupled donkey anti-rabbit Ig or /H9251 (PBST; all subsequent incubations were performed in the same solution) blocked with 2% nonfat dry milk in PBS containing 0.5% Tween-20.

**Antibodies and Western blotting analysis**

C. Each survival curve represents and 2003), with mortality counts every 12 h. To induce expression of UAS-Avdulov, S., Li, S., Michalek, V., Burrichter, D., Peterson, M., Perlman, Dian Institute of Health Research (CIHR) to P.L. and N.S. N.S. is a CIHR stocks. This work was supported by an operating grant from the Canada

**Acknowledgments**

We thank Deborah Kimbrell, Martin Jünger, and Ernst Hafen for fly stocks. This work was supported by an operating grant from the Canadian Institute of Health Research (CIHR) to P.L. and N.S. N.S. is a CIHR International Scholar.

**References**

Avdulov, S., Li, S., Michalek, V., Burrichter, D., Peterson, M., Perlman, D.M., Manivel, J.C., Sonenberg, N., Yee, D., Bitterman, P.B., et al. 2004. Activation of translation complex elf4F is essential for the genesis and maintenance of the malignant phenotype in human mammary epithelial cells. *Cancer Cell* 5:553–563.

Bernal, A. and Kimbrell, D.A. 2000. *Drosophila Thor* participates in host immune defense and connects a translational regulator with innate immunity. *Proc. Natl. Acad. Sci.* 97: 6019–6024.

Bernal, A., Schoenfeld, R., Kleinhesslinsk, K., and Kimbrell, D. 2004. Loss of Thor, the single 4E-BP gene of *Drosophila*, does not result in lethality. *Drosoph. Inf. Serv.* 87: 81–84.

Bjornsti, M.A. and Houghton, P.J. 2004. Lost in translation: Dysregulation of cap-dependent translation and cancer. *Cancer Cell* 5:519–523.

Brand, A.H. and Perrimon, N. 1993. Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* 118: 401–415.

Colombani, J., Raisin, S., Pantalacci, S., Radimerski, T., Montagne, J., and Léopold, P. 2003. A nutrient sensor mechanism controls *Drosophila* growth. *Cell* 114: 739–749.

Finger, D.C., Salama, S., Tsou, C., Harlow, E., and Blenis, J. 2002. Mammalian cell size is controlled by mTOR and its downstream targets S6K1 and 4EBP1/elf4E. *Genes & Dev.* 16: 1472–1487.

Giannakou, M.E., Goss, M., Jünger, M.A., Hafen, E., Leevers, S.J., and Partridge, L. 2004. Long-lived *Drosophila* with overexpressed dFOXO in adult fat body. *Science* 305: 361.

Gingras, A.C., Raught, B., and Sonenberg, N. 1999. elf4F initiation factors: Effectors of mRNA recruitment to ribosomes and regulators of translation. *Annu. Rev. BioChem.* 68: 913–963.

Grolloeau, A., Bowman, J., Pradet-Balade, B., Puravs, E., Hanash, S., Garcia-Sanz, J.A., and Beretta, L. 2002. Global and specific translational control by rapamycin in T cells uncovered by microarrays and proteomics. *J. Biol. Chem.* 277: 22175–22184.

Haghighat, A., Mader, S., Pause, A., and Sonenberg, N. 1995. Repression of cap-dependent translation by 4E-binding protein 1: Competition with p220 for binding to eukaryotic initiation factor 4E. *EMBO J.* 14: 5701–5709.

Hay, N. and Sonenberg, N. 2004. Upstream and downstream of mTOR.

**Role of 4E-BP in metabolic stress response**

*Genes & Dev.* 18: 1926–1945.

Holcik, M., Sonenberg, N., and Korneluk, R.G. 2000. Internal ribosome initiation of translation and the control of cell death. *Trends Genet.* 16: 469–473.

Hwangbo, D.S., Gershman, B., Tu, M.P., Palmer, M., and Tatar, M. 2004. *Drosophila* dFOXO controls lifespan and regulates insulin signalling in brain and fat body. *Nature* 429: 562–566.

Jia, K., Chen, D., and Riddle, D.L. 2004. The TOR pathway interacts with the insulin signaling pathway to regulate *C. elegans* larval development, metabolism and life span. *Development* 131: 3897–3906.

Johnstone, O. and Lasko, P. 2001. Translational regulation and RNA localization in *Drosophila* oocytes and embryos. *Annu. Rev. Genet.* 35: 365–406.

Jünger, M.A., Rintelen, F., Stocker, H., Wasserman, J.D., Vegh, M., Radimerski, T., Greenberg, M.E., and Hafen, E. 2003. The *Drosophila* Forkhead transcription factor FACTO mediates the reduction in cell number associated with reduced insulin signaling. *J. Biol.* 2: 30.

Kapahi, P., Zid, R.M., Harper, T., Koslover, D., Sapin, V., and Benzer, S. 2004. Regulation of lifespan in *Drosophila* by modulation of genes in the TOR signaling pathway. *Curr. Biol.* 14: 885–890.

Li, S., Sonenberg, N., Gingras, A.C., Peterson, M., Avdulov, S., Polunovsky, V.A., and Buttemer, F.B. 2002. Translational control of cell fate: Availability of phosphorylation sites on translational repressor 4E-BP governs its proapoptotic potency. *Mol. Cell Biol.* 22: 2853–2861.

Mader, S. and Sonenberg, N. 1995. Cap binding complexes and cellular growth control. *Biochimie* 77: 40–44.

Miron, M., Verdu, J., Lachance, P.E., Birnbaum, M.J., Lasko, P.F., and Sonenberg, N. 2001. The translational inhibitor 4E-BP is an effector of PI3K/Akt signalling and cell growth in *Drosophila*. *Nat. Cell Biol.* 3: 596–601.

Patel, J., McLeod, L.E., Vries, R.G., Flynn, A., Wang, X., and Proud, C.G. 2002. Cellular stresses profoundly inhibit protein synthesis and modulate the states of phosphorylation of multiple translation factors. *Eur. J. Biochem.* 269: 3076–3085.

Puig, O., Marr, M.T., Ruhl, M.L., and Tjian, R. 2003. Control of cell number by *Drosophila* FOXO: Downstream and feedback regulation of the insulin receptor pathway. *Genes & Dev.* 17: 2006–2020.

Rodriguez, A., Zhou, Z., Tang, M.L., Meller, S., Chen, J., Bellen, H., and Kimbell, D.A. 1996. Identification of immune system and response genes, and novel mutations causing melanotic tumor formation in *Drosophila melanogaster*. *Genetics* 143: 929–940.

Schmelzle, T. and Hall, M.N. 2000. TOR, a central controller of cell growth. *Cell* 103: 253–262.

Sonenberg, N., Hershey, J.W.B., and Mathews, M. 2000. Translational control of gene expression. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

van der Velden, A.W. and Thomas, A.A. 1999. The role of the 5′ untranslated region of an mRNA in translation regulation during development. *Int. J. Biochem. Cell Biol.* 31: 87–106.

Wang, M.C., Bohnmann, D., and Jasper, H. 2005. JNK extends life span and limits growth by antagonizing cellular and organism-wide responses to insulin signaling. *Cell* 121: 115–125.

Waskiewicz, A.J., Flynn, A., Proud, C.G., and Cooper, J.A. 1997. Mitogen-activated protein kinases activate the serine/threonine kinases Mnk1 and Mnk2. *EMBO J.* 16: 1909–1920.

Zinke, I., Schütz, C.S., Katzenberger, J.D., Bauer, M., and Pankratz, M.J. 2002. Nutrient control of gene expression in *Drosophila*: Microarray analysis of starvation and sugar-dependent response. *EMBO J.* 21: 6162–6173.
Starvation and oxidative stress resistance in *Drosophila* are mediated through the eIF4E-binding protein, d4E-BP

Gritta Tettweiler, Mathieu Miron, Mark Jenkins, et al.

*Genes Dev.* 2005, 19: Access the most recent version at doi:10.1101/gad.1311805

Supplemental Material

http://genesdev.cshlp.org/content/suppl/2005/07/29/gad.1311805.DC1

References

This article cites 29 articles, 12 of which can be accessed free at:

http://genesdev.cshlp.org/content/19/16/1840.full.html#ref-list-1

License

Email Alerting Service

Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or click here.

---

**Boost NGS microRNA profiling.**

Read about 3 methods tested

EXIQON

New a QIAGEN company

---

Cold Spring Harbor Laboratory Press