Of FOXes and forgetful worms

Nazif Alic
Institute of Healthy Ageing,
University College London,
WC1E 6BT, London, United Kingdom,
Email: n.alic@ucl.ac.uk

Abstract

Age-related cognitive decline is one of the most haunting aspects of human aging. In a recent publication, Coleen Murphy and colleagues describe the transcriptional program that maintains youthful function of aging neurons in the nematode worm.

Main body

For many of us, old age will be marked by deteriorating cognitive performance, ranging from slightly disconcerting difficulties in remembering a telephone number to highly debilitating and progressive dementias, such as Alhzeimer's (Morrison and Baxter, 2012). Such cognitive impairments are often the most devastating aspect of human aging on a personal level. The alarming increase in the proportion of elderly individuals in our societies has fueled the search for interventions to ameliorate health in older humans. In a recent publication, a Princeton University research group led by Coleen Murphy describes the transcriptional circuit that acts to improve the performance of aging neurons in the nematode worm, *Caenorhabditis elegans*, maintaining youthful capacity for learning, memory and axon regeneration in older individuals (Kaletsky et al., 2016).

Aging is a complex phenomenon, yet we now know that it can be modulated by a growing number of environmental, pharmacological or genetic interventions. Reduction in the signals propagated through the nutrient sensing pathways, such as the insulin/IGF-signaling pathway, can extend animals' lifespan and improve health in old age. Dampening of insulin-like signals remodels the transcriptional landscape by activating the Forkhead Box

O (FoxO) class of transcription factors to promote health and survival in old age (Murphy et al., 2003). Indeed, FoxOs are evolutionarily conserved longevity determinants, and *FoxO3* is one of two genes consistently associated with human longevity (Morris et al., 2015). Hence, understanding transcriptional regulation by FoxOs may hold the key to a healthy old age.

Similar to the age-related cognitive decline in humans, nematode worms lose their capacity to learn and remember as they age, while their neuronal cells, like their human counterparts, lose their ability to regenerate axons after injury. These deficits can be improved by activation of the sole FoxO ortholog present in the worm: DAF-16 (Byrne et al., 2014; Kauffman et al., 2010). But how does DAF-16 do this? Previous work has characterized DAF-16-driven transcriptional changes in whole worms, defining genes important in longevity (Murphy et al., 2003); however, these can mostly be mapped to the worm's intestine and hypodermis, leaving it unclear how DAF-16 acts in neurons to preserve their long-term functioning.

To answer this question, Kaletsky, Lakhina and colleagues isolated adult neurons and determined their transcript profiles, charting a transcriptional landscape distinct from larval and embryonic neuronal transcriptomes. Characterizing the transcriptional changes caused by the activation of DAF-16, the researchers show DAF-16 neuronal targets to be distinct from the previously described, whole-worm targets. In non-neuronal tissues, DAF-16-regulated genes predominantly encode metabolic functions, while in adult neurons, DAF-16 regulates neuron-specific genes, such as serpentine, neuropeptide and octopamine receptors (Kaletsky et al., 2016; Murphy et al., 2003). This cell-type specificity is an emerging pattern in FoxO biology: FoxOs target genes suitable for functional maintenance of the specific cells in which FoxOs are activated.

Importantly, the researchers used functional assays to demonstrate that neuronal DAF-16 targets are required for enhanced memory or improved axonal regeneration when insulin-like signaling is inhibited. Similar to the effect of non-neuronal DAF-16 targets on longevity, concerted regulation of a number of neuronal genes appears to contribute to the functional outcome. The broad network of memory-extending genes engaged by activated DAF-16 is already expressed in wild-type neurons. Thus, DAF-16 appears to preserve

normal neuronal function rather than compensate through an alternative mechanism.

The neuronal DAF-16 transcriptional network is complex: the results of Kaletsky, Lakhina and colleagues suggest that, in adult worm neurons, DAF-16 mobilizes up to 60 other transcription factors to indirectly drive the transcriptional changes instigated by insulin reduction. As part of this intricate web of transcriptional regulators, the study identified another forkhead transcription factor: FKH-9. DAF-16 binds directly to the *fkh-9* promoter to activate *fkh-9* transcription. Interestingly, *fkh-9* is required not only in neurons to maintain their health, but also in non-neuronal tissues to achieve the longevity in an insulin-signaling mutant (**Figure 1**). A detailed investigation of its interplay with DAF-16 and the evolutionary conservation of neuro-protective potential of FKH-9 is now warranted.

Neurons in adult *C. elegans* are terminally differentiated, post-mitotic cells. In this respect, the current study complements previous work on the function of FoxO factors in the mouse. In mammals, neural stem cells in defined brain regions can self-renew and generate most of the cell lineages constituting the mammalian nervous system, ensuring its function and homeostasis. FoxOs safegard the maintenance of these cells in adult mice by constraining their proliferation (Chen and Finkel, 2009). Furthermore, a FoxO factor (FoxO6) is required for memory consolidation in mice, transcriptionally regulating genes involved in synaptic function (Salih et al., 2012). Hence, FoxOs may act in both terminally differentiated neurons and the neural stem cells to maintain healthy nervous system performance with age.

Kaletsky, Lakhina and colleagues identify a transcriptional program that enhances neuronal health in aging worms, thus opening promising avenues towards improved cognitive performance in older humans. Their findings may be also relevant in a disease context. In humans, age is the main risk factor for dementia. The etiology of many neurodegenerative diseases includes the accumulation of toxic protein aggregates, and aging neurons may be particularly sensitive to such insults. Indeed, age of exposure is an independent factor increasing vulnerability to toxic peptides in a fly model of Alhzeimer's disease (Rogers et al., 2012). DAF-16 is already known to counteract toxic protein aggregation (Cohen et al., 2006). It would be

interesting to examine whether the mechanisms whereby activated DAF-16 helps aging neurons maintain youthful function can be exploited for treatment or prevention of neurodegenerative diseases.

As we build our understanding of how FoxOs preserve health in old age, it is important to finely delineate the extent of evolutionary conservation of FoxO longevity programs. We should continue exploring the complexity of transcriptional regulation by FoxOs, aiming at a holistic understanding of all the players involved and their interactions, mapping the longevity-assuring transcriptional networks within specific cell types as well as entire organisms.

Acknowledgements

NA's laboratory is supported by the BBSRC and the Royal Society.

Figure caption

Figure 1. DAF-16 and FKH-9 in neuronal health and lifespan. DAF-16 and FKH-9 act in neurons to promote neuronal health while they are also required in non-neuronal tissues to enhance lifespan under conditions of reduced insulin-like signaling. DAF-16 directly regulates transcription of *fkh-9*, in at least some cell types. More speculative links are indicated by thinner lines.

References

Byrne, A.B., Walradt, T., Gardner, K.E., Hubbert, A., Reinke, V., and Hammarlund, M. (2014). Insulin/IGF1 signaling inhibits age-dependent axon regeneration. Neuron *81*, 561-573.

Chen, E., and Finkel, T. (2009). Preview. The Tortoise, the hare, and the FoxO. Cell Stem Cell *5*, 451-452.

Cohen, E., Bieschke, J., Perciavalle, R.M., Kelly, J.W., and Dillin, A. (2006). Opposing activities protect against age-onset proteotoxicity. Science *313*, 1604-1610.

Kaletsky, R., Lakhina, V., Arey, R., Williams, A., Landis, J., Ashraf, J., and Murphy, C.T. (2016). The *C. elegans* adult neuronal IIS/FOXO transcriptome reveals adult phenotype regulators. Nature *529*, 92-96.

Kauffman, A.L., Ashraf, J.M., Corces-Zimmerman, M.R., Landis, J.N., and Murphy, C.T. (2010). Insulin signaling and dietary restriction differentially influence the decline of learning and memory with age. PLoS Biol *8*, e1000372.

Morris, B.J., Willcox, D.C., Donlon, T.A., and Willcox, B.J. (2015). FOXO3: A Major Gene for Human Longevity--A Mini-Review. Gerontology *61*, 515-525.

Morrison, J.H., and Baxter, M.G. (2012). The ageing cortical synapse: hallmarks and implications for cognitive decline. Nature reviews. Neuroscience *13*, 240-250.

Murphy, C.T., McCarroll, S.A., Bargmann, C.I., Fraser, A., Kamath, R.S., Ahringer, J., Li, H., and Kenyon, C. (2003). Genes that act downstream of DAF-16 to influence the lifespan of *Caenorhabditis elegans*. Nature *424*, 277-283.

Rogers, I., Kerr, F., Martinez, P., Hardy, J., Lovestone, S., and Partridge, L. (2012). Ageing increases vulnerability to abeta42 toxicity in *Drosophila*. PLoS One *7*, e40569.

Salih, D.A., Rashid, A.J., Colas, D., de la Torre-Ubieta, L., Zhu, R.P., Morgan, A.A., Santo, E.E., Ucar, D., Devarajan, K., Cole, C.J., et al. (2012). FoxO6 regulates memory consolidation and synaptic function. Genes Dev *26*, 2780-2801.