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NEWS AND VIEWS

Ageing in worms: N-acylethanolamines take control

Run Shen ✉

The Salk Institute for Biological Studies, 10010 North Torrey Pines Road, La Jolla, CA 92037, USA
✉ Correspondence: rshen@salk.edu

Studies of dietary restriction's effect on lifespan extension in *C. elegans* and other model organisms have greatly expanded our knowledge about genetic network underlying stress response, ageing, and ageing related diseases. Among key molecules and signaling pathways implicated are Foxo (*daf-16*) and Foxa (*pha-4*) transcriptional factors and the nutrient sensing insulin/TOR pathway (Wolkow et al., 2000; Panowski et al., 2007; Hansen et al., 2008). However, details about how diet-derived metabolic signals connect nutrient availability to ageing are largely unknown. Recently, a group led by Mathew Gill discovered that lipid derived N-acylethanolamines (NAEs) respond to dietary restriction and are both necessary and sufficient to control lifespan in worms (Lucanic et al., 2011). This finding is as much revealing as it is surprising, because the endocannabinoid system, to which NAEs belong, is believed to operate only in higher organisms (McPartland et al., 2006). It also underscores the power of model organisms such as the lowly earth worm in dissecting the molecular mechanisms controlling ageing.

NAEs are a type of compounds formed by linking certain acyl groups to the nitrogen of ethanolamine. Bioactive N-acylethanolamines bind and activate the cannabinoid receptor 1 (CB1), which also binds and is activated by tetrahydrocannabinol (THC), the major psychotropic component in marijuana. The first two of the endogenous CB1 ligands purified are N-arachidonoyl ethanolamine (anandamide) and 2-arachidonoyl glycerol (2-AG) (Di Marzo and Matias, 2005). The endocannabinoid system is essential for controlling food intake and maintaining energy balance in mammals, but whether it plays any direct role in dietary restriction mediated lifespan extension is not known until this current study.

To find out more about how dietary signals control ageing, Gill and colleagues set out to ask if *C. elegans* has an active endocannabinoid system in play. They identified a set of NAEs, including eicosapentaenoyl ethanolamide (EPEA) and arachidonoyl ethanolamide (AEA) in *C. elegans*. In mammals, NAE levels are controlled by N-acyl-phosphatidylethanolamine-specific phospholipase D (NAPE-PLD), which controls the last step of biosynthesis of NAEs, and fatty acid amide hydrolase (FAAH), which inactivates NAE molecules via hydrolysis. By manipulating FAAH expression in worms, Gill and colleagues could effectively detect changes in NAE levels, suggesting that a conserved pathway regulating NAEs exists in worms. Nutrient availability directly controls animal growth and reproductive development. To examine a possible role of NAEs in this context, Gill and colleagues altered the levels of endogenous and exogenous NAEs and found that NAEs regulate reproductive growth and dauer formation. This finding begs the questions of how endogenous NAE levels are regulated, and if they play a role in lifespan regulation. Interestingly, they found that the level of endogenous NAEs decreases in response to dietary restriction but recovers when the animals are refed. Then, the question is whether decreased NAE levels may partly mimic dietary restriction and extend lifespan. And they found reducing endogenous NAEs by *faah-1* overexpression extended lifespan in the presence of abundant food but not under optimal dietary restriction, suggesting this *faah-1* overexpression meditated effect shares some common mechanism with dietary restriction. To further define the function of NAEs in the genetic network underlining lifespan extension by dietary restriction, Gill and colleagues interrogated the interactions among *faah-1*, *daf-16,* and pha-4. *daf-16* has been extensively studied and is known to upregulate gene expression favoring lifespan extension including superoxide dismutases (SODs) and autophagy (Hansen et al., 2008). Transcription of *daf-16* is negatively regulated by insulin/Akt signaling. Lifespan extension mediated by *faah-1* overexpression is not dependent on *daf-16* function, whereas *Pha-4*, a transcriptional factor promoting longevity in worms, is required. Since reducing NAE levels was shown to be sufficient to extend lifespan, it would be interesting to ask if elevating NAE levels would

suppress stress resistance and lifespan extension. To their prepared minds, exogenous NAE treatment extended not only the lifespan of wild type control, but also *daf-2* mutants. The exogenous NAEs' effect on *daf-2* mutants is consistent with previous studies showing that insulin/Foxo function is dispensable for NAE signaling. Also, consistent is that elevating NAE levels did not decrease lifespan under high food in-take conditions since endogenous NAE response in this case had much less potential to be upregulated compared with dietary restriction.

Up until this finding by Gill and colleagues, the endocannabinoid system is not known to exist in *C. elegans*. In mammals, endocannabinoids are known to regulate energy balance by modulating the hypothalamic circuitry controlling food intake and energy expenditure. Several hypothalamic and orexigenic mediators may be the targets, including corticotrophin-releasing hormone (CRH), melanin-concentrating hormone and prepro-orexin. In peripheral tissues, endocannabinoids and receptors are also present, particularly in the adipose tissue, to regulate adipogenesis, glucose uptake, lipogenesis and fat storage (Di Marzo and Matias, 2005). Yet, little is known about how this system regulates stress resistance and ageing. In Gill and colleague's study, genetic epistasis analysis places NAEs downstream of *daf-2*/*daf-16*, but upstream of *pha-4*, which has been proposed to regulate lifespan through upregulating expression of *sod-2* and *sod-4* (Panowski et al., 2007). The decrease of NAEs associated with dietary restriction may correlate with high levels of *sod*s, which helps to explain the role of NAEs in lifespan regulation in worms. The endocannabinoid system in human has been implicated in the pathophysiology of ischemic stroke and neurodegenerative diseases of immune origin, including multiple sclerosis (Howlett et al., 2006). So, the study by Gill and colleagues may enhance our understanding of these diseases in humans, given the connection it begins to establish between the endocannabinoid system and ageing. On the other hand, studies in mammals may also provide insight to elucidate the mechanism of endocannabinoid signaling in worms. Peroxisome proliferator-activated receptors (PPARs) are a family of nuclear receptors that, upon activating ligand-binding, act as transcription factors to regulate gene expression for lipid metabolism. An increasing body of evidence suggests that the ability to bind PPARs is an attribute common to many endocannabinoids and is of physiological relevance (Fu et al., 2003). Since it appears that worms lack clear orthologues of cannabinoid receptors, PPARs may take on the task of relaying endocannabinoid signals. Additionally, this study establishes a powerful genetic system for future research on the roles of endocannabinoid system. The transparent body and low redundancy in gene functions, coupled with the ease of applying the power of RNAi screen, make *C. elegans* an advantageous system for elucidating the mechanism of endocannabinoid system and generating potential targets for drug design. Even better, since automated high-content live animal drug screening has been developed for worms, now compound libraries can be applied directly to target specific reporters or phenotypes (Gosai et al., 2010).

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