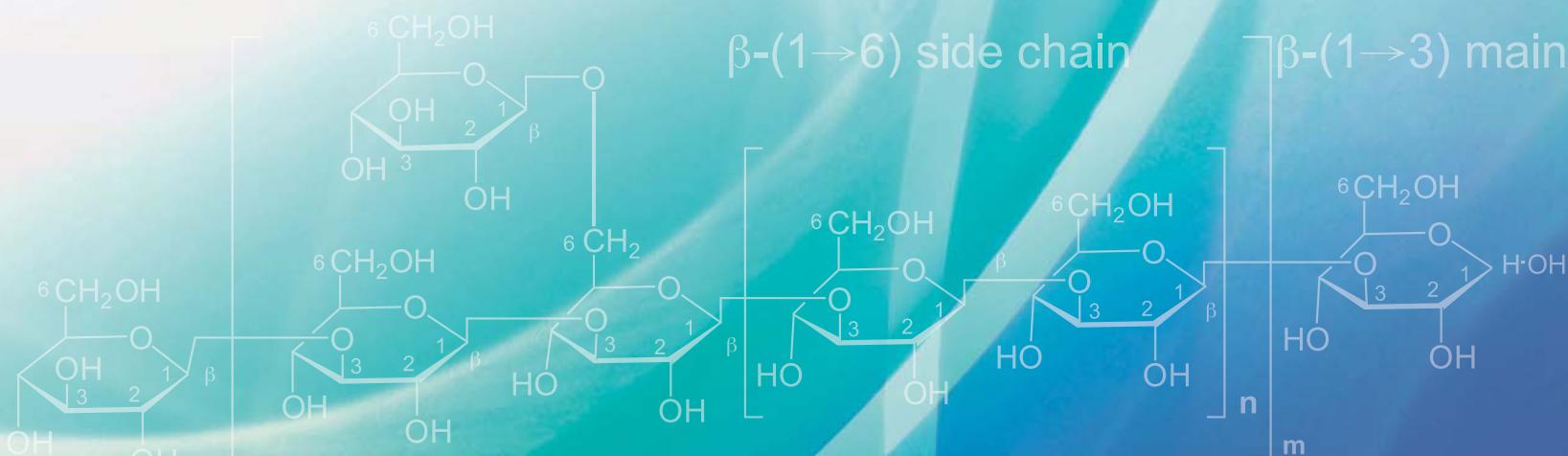


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Dietary Supplements May Increase Longevity by Up-regulation of Daf-16/FoxO Gene



By

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Dietary Supplements May Increase Longevity by Up-regulation of Daf-16/FoxO Gene

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Introduction

Gene modification of *daf-16* (the equivalent of mammalian FoxOs) in nematode *C. elegans* has been shown to double the expected lifespan[1]. Mammals have four isoforms of the FoxO transcription factor family, FoxO1, FoxO3, FoxO4 and FoxO6. Research data from nested, case-control *Hawaii life span study* provided support for a genetic basis in the aging process, mainly a role of FoxO3A in longevity[2]. This observation has been confirmed in other populations as well, including Japanese, German, American, Italian, and Chinese[3]. It is now known that insulin and growth factor inhibit FoxO factors, while stress stimuli such as DNA damage, nutrient deprivation, cytokines and hypoxia up-regulate FoxO factors. FoxO-dependent transcription plays an important role in glucose metabolism, angiogenesis, detoxification of reactive oxygen species (ROS), repair of damaged DNA, apoptosis, stem cell maintenance, stress resistance, as well as immune, muscular and neuronal functions. Even though aging is a fundamental process with great innate diversity, we now know that aging, like many other biological process, is subject to regulation by pathways that have been conserved during evolution[4,5]. Recent advances in genetic and biochemical studies have made elucidation of these complex biological pathways and processes possible.

Objectives

In this study we aim to review potential of dietary factors with significant effects on modulating cellular transcription of *daf-16* with implication to modify aging in this model system.

Methods

Systemic search of the literature was conducted in electronic databases of MEDLINE. The reference lists of all papers and our files were searched for relevant publications. Experts in the field and manufacturers of dietary supplements were contacted for published and unpublished data. No language restrictions were imposed.

Results

Calorie restriction (CR) without malnutrition is known to delay aging and extends life span up to 50% in diverse species, including *C. elegans*, yeast, flies fish, rodent, and mammals[6]. A 20-year longitudinal adult-onset CR study in rhesus monkeys showed moderate CR not only lowered the incidence of aging related deaths, it also reduced the incidence of diabetes, cancer, cardiovascular disease, and brain atrophy[7]. A reduction of calories 30-50% below ad libitum levels of a nutritious diet can increase lifespan[8]. Clinical trials have been initiated to study whether such intervention is relevant to human aging. However, the utility of CR would be hampered because of the degree and length of restriction required. Identification of compounds that mimic CR effects by targeting metabolic and stress response pathways affected by CR without substantially restricting caloric intake offers great potential in aging and disease prevention. In rodent model, both nutritional and hormonal factors can affect the gene expression of FoxOs. For example, fasting 48 h significantly elevated mRNA levels of FoxO1 (1-5 folds), FoxO3 (1-4 folds), and FoxO4 (1-6 folds), while daily administration of dexamethasone for 1 week increases FoxO1 (1-8 folds) and FoxO3 (2-4 folds) in rats[9]. Muscle biopsies have shown that resistance exercises have age related alteration in FoxO3A signaling and expression in healthy young (24 + YO) and old (65-85 YO) female volunteers[10].

Resveratrol, a polyphenolic compound found in red wine, berries, grapes and peanuts was associated with slight lifespan increases (10%) in *C. elegans*, although it did not extend lifespan in flies in one study and in mice on a normal diet[6]. Feeding mice a diet supplemented with resveratrol protects them against toxicity associated with high-fat diet, enhances metabolic function and leads to gene expression changes similar to those caused by dietary restriction, namely Sir2 activation. However, this hypothesis has proven controversial, and several groups have reported Sir2-independent lifespan extension by dietary restriction in both yeast and *C. elegans*[11].

Various natural substances and vitamin supplements were evaluated for their possible effects on longevity using *C. elegans* model[12]. The study data showed acetic acid (vinegar) extended *C. elegans* lifespan by 25%, while Vitamins C and E, and vitamin B complex showed less significant effect (<3%).⁶ Mushroom extracts, including Reishi F-3 polysaccharide (*Ganoderma lucidum*), mycelium fractions of Chang-Chih (*A. camphorata*), and lion's mane (*H. erinaceus*) were associated with significant increase of lifespan, ranging from 25% to 35%. A synergistic, life-extending effect was found when acetic acid and RF-3 were combined (40%)[12].

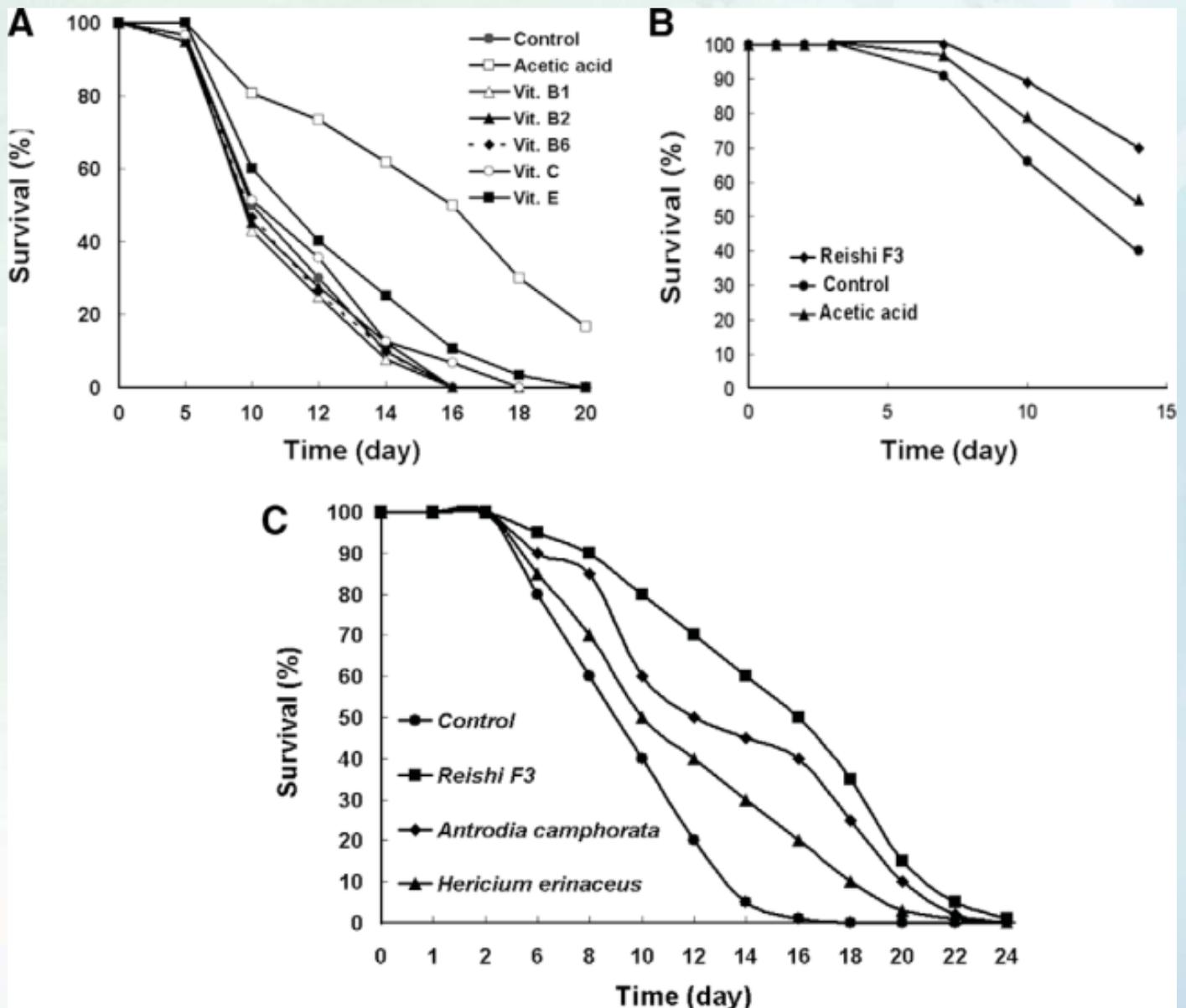


Figure 1. Effects of natural supplements and herbal mushrooms on the lifespan of *C. elegans*. The curves shown represent changes in percentage survival worms (at least 60 worms were initially transferred into each of three wells for triplicate assays) at different times (days) after treatments with each individual supplement. (A) Worms treated with water (control), acetic acid (50 ppm), vitamin B1 (50 ppm), vitamin B2 (50 ppm), vitamin B6 (50 ppm), vitamin C (50 ppm), and vitamin E (50ppm). (B) Worms treated with water (control), acetic acid (50 ppm) and RF3 (100 ppm). (C) Worms treated with water (control), RF3 (100 ppm) and crude extracts of *A. camphorata* (100 ppm) and *H. erinaceus* (100 ppm) herbal mushrooms. Lifespan assays were determined at 20 °C. Percentage survival was calculated from the number of living worms at indicated time periods(days) as compared with the original number of testing worms. P values were also calculated for each set of lifespan assay, which represented a direct comparison between the control group and each experimental treatment group during the same period. These assays were carried out in triplicate and analyzed statistically with *P <0.01 for each set of experiments.[12]

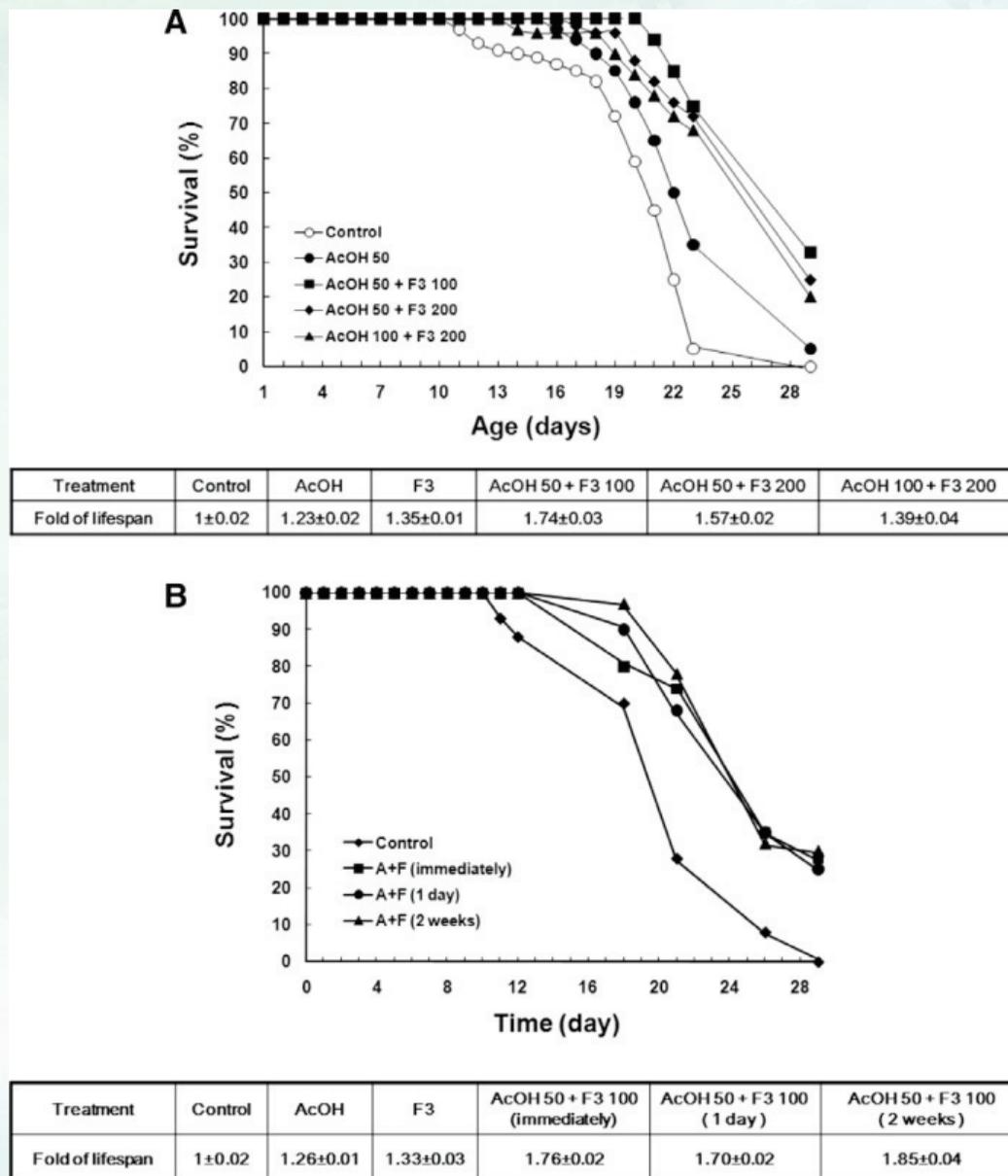


Figure 2. The cumulative effect of RF3 and acetic acid on lifespan enhancement of *C. elegans*. (A) Lifespan of *C. elegans* treated with mixtures of RF3 (F3) and acetic acid (AcOH) in different concentrations. The best result was achieved with a mixture containing 50 ppm AcOH and 100 ppm F3 (■) as compared with (1) control (○), (2) 50 ppm AcOH (●), (3) 50 ppm AcOH + 200 ppm F3 (◆), (4) 100 ppm AcOH + 200 ppm F3 (▲). (B) Effect of the mixture of RF3 and acetic acid on lifespan of *C. elegans* after storage for different periods. Control (◆), test after immediate mixing (■), test after mixing for 1 day (●), and test after mixing for 2 weeks (▲). The results show that long-term storage (up to two weeks) of the mixture of RF3 and acetic acid did not affect the efficacy on the lifespan extension of treated *C. elegans*. All tables under the survival curves refer to the lifespan extension induced by the mixture as compared with the control group. All the values shown are mean ± SD, calculated from triplicate experiments.

Discussion

Calorie restriction has the remarkable ability to extend lifespan and health span. Studies have shown the low-energy sensing AMP-activated protein kinase AMPK/aak-2 and the Forkhead transcription factor FoxO/*daf-16* are necessary for longevity induced by calorie restriction[6]. Resveratrol extends lifespan in an AMPK-dependent, but FoxO-independent manner. Activation of AMPK is not always coupled to that of FoxO, even though AMPKs ability to extend lifespan is dependent on the presence of FoxO[6]. On the other hand, glucose shortens the lifespan of *C. elegans* (20%) by down-regulating *daf-16*/FoxO activity and aquaporin gene expression[13]. Natural substances, such as Chang-Chih mushroom extract and Reishi F-3 polysaccharide extract were shown to up-regulate *daf-16* expression via TIR-1 and other MAPK pathway, distinct from the well-known conserved insulin/IGF-1 *daf-2* signaling pathway inhibited by acetic acid and lion's mane extract, resulting in increased expression of the longevity factor *daf-16*. The lifespan extension observed was more dramatic in worms fed with Reishi F-3 than with Chang-Chih mushroom (*A. camphorata*) extract[12]. These results suggest that different ways of manipulating nutrients and chemical compounds may extend lifespan via independent and nonlinear genetic pathways, and a well designed combination may add synergistic effects.

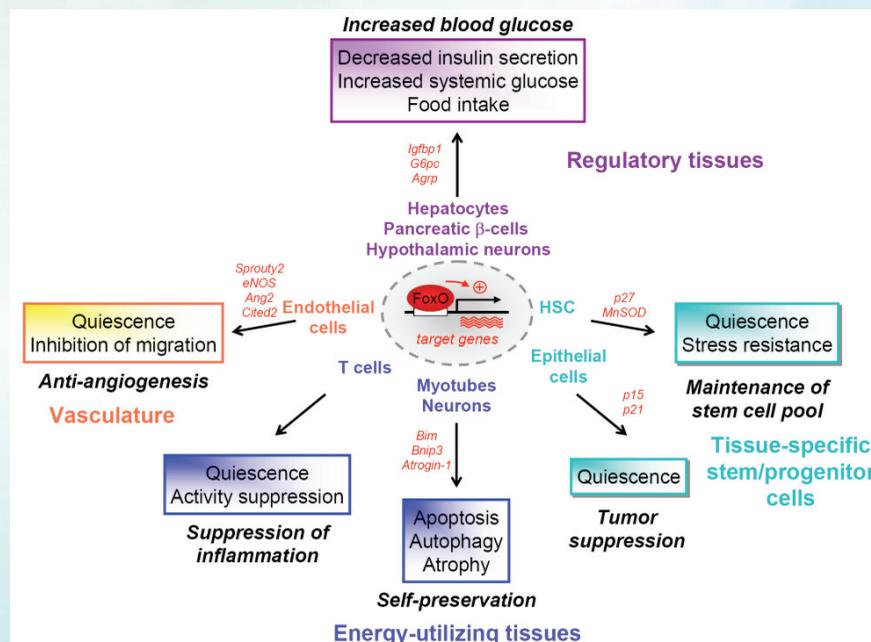


Fig. 3. Role of FoxO transcription factors in tissue homeostasis

FoxO-dependent transcription serves to increase systemic glucose levels by regulating gluconeogenesis, insulin secretion, and food intake in the regulatory cells of the organism such as the hepatocytes, pancreatic β -cells, and hypothalamic neurons. In energy-utilizing cells, such as myocytes and neurons, the FoxO factors appear to be activated in response to harsh environment conditions such as starvation and oxidative stress to initiate atrophy, autophagy or apoptosis. In T cells, FoxO factors attenuate proliferation and hyper-activation. In the endothelial cells of the vascular system, the FoxO factors regulate genes to suppress proliferation and inhibit migration. FoxO factors also display a crucial role in the maintenance of hematopoietic stem cells (HSC) by coordinating quiescence, stress resistance and terminal differentiation. Overall, FoxO factors appear to promote an organismal metabolic shutdown in response to harsh environmental conditions, such as starvation, to enable the organism to survive in anticipation of improved environmental conditions.[4]

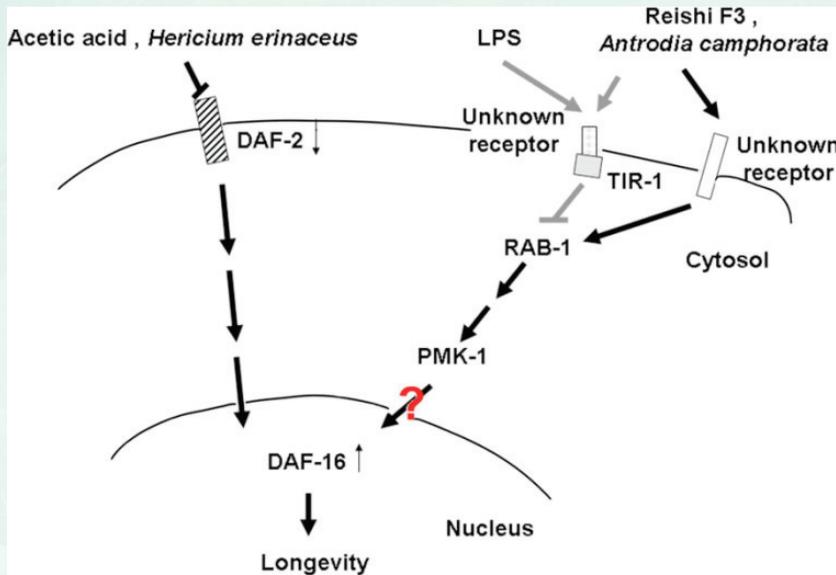


Figure 4. The proposed signaling pathways for *daf-16* mediated longevity in *C. elegans* by acetic acid, RF3, extracts of *A. camphorata* and *H. erinaceus*. RF3 probably attaches to at least two different types of receptors on the cell surface, one increased *tir-1* expression leading to downstream signal transduction to reduce *rab-1* expression, and the other probably bind to another unknown receptor, which could induce *rab-1* expression followed by activation of the MAPK pathway, resulting in an increase of transcription factor *daf-16* activity to

extend the lifespan. The longevity-promoting effect of acetic acid and *H. erinaceus* was probably mediated by a pathway reducing the trans-membrane receptor *daf-2* expression, indirectly increased the downstream expression of *daf-16*. The upward and downward arrows in thin line show the pathways leading to elevated or reduced expression levels of some key factors like DAF-2 and DAF-16, and the question mark (?) some unidentified factors or routes between PMK-1 and DAF-16. [12]

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Disclosures

The authors declare no commercial or financial relationships that could be constructed as a potential conflict of interest. Dr. Deborah Lin is scientific advisor for Wyntek Corporation and chief medical consultant for Wynlife Healthcare Inc. San Diego, CA.

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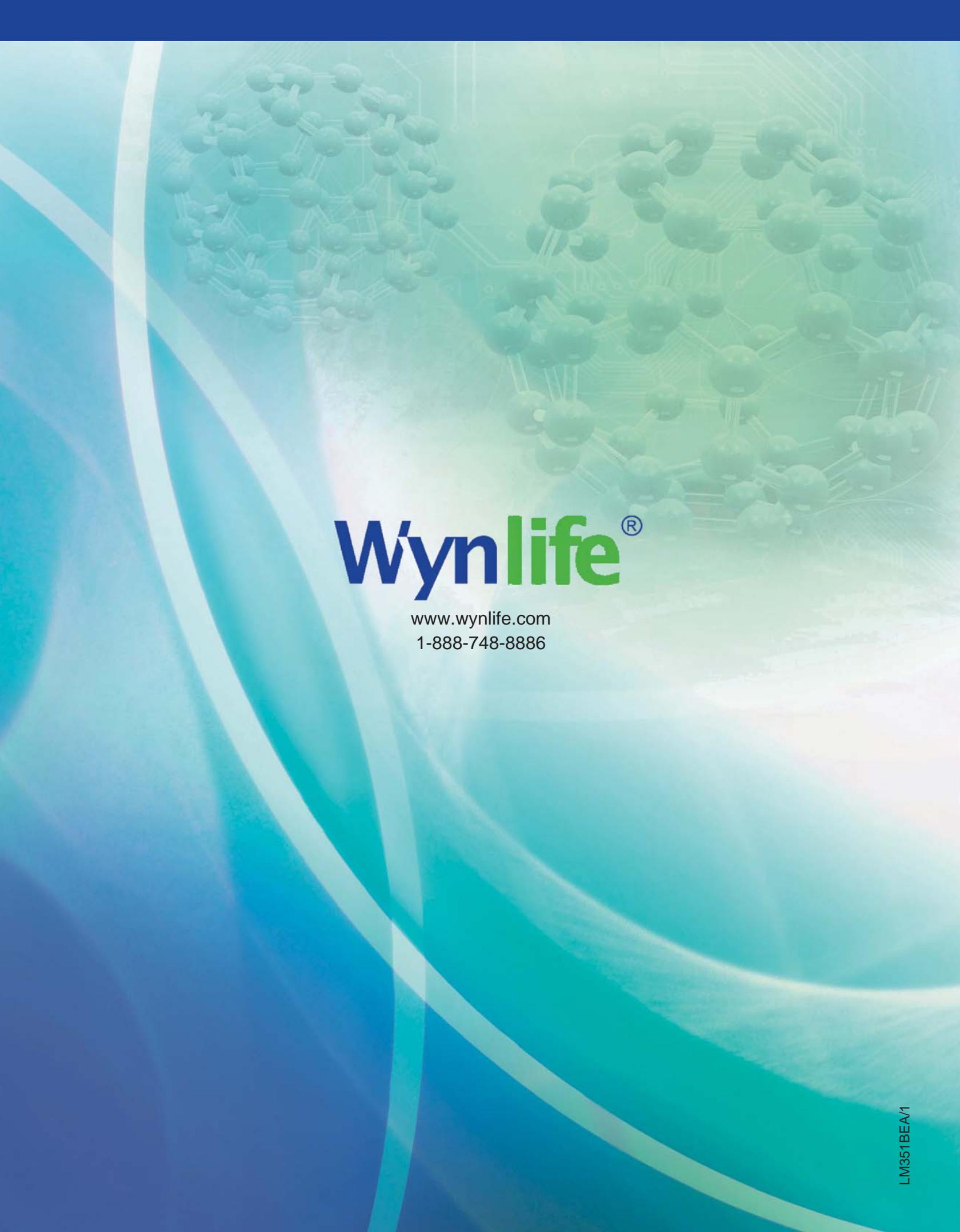
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