ATF4 activity: a common feature shared by many kinds of slow-aging mice

Weiquan Li, Xinna Li and Richard A. Miller

Summary

ATF4, a DNA-binding factor that modulates responses to amino acid availability and ribosomal function, has been shown to be altered in both liver and fibroblasts from two strains of long-lived mice, i.e. Snell dwarf and PAPP-A knockout mice. New data now show elevated ATF4 levels, and elevation of ATF4-dependent proteins and mRNAs, in liver of mice treated with acarbose or rapamycin, calorically restricted mice, methionine-restricted mice, and mice subjected to litter crowding. Elevation of ATF4, at least in liver, thus seems to be a shared feature of diets, drugs, genes, and developmental alterations that extend maximum lifespan in mice.

Key words: acarbose; caloric restriction; longevity; methionine restriction; rapamycin.

Introduction

ATF4 is a transcriptional factor which senses deficits in protein translation, typically related to ER stress or amino acid limitation, and in turn activates a group of target genes through a consensus ATF4 promoter binding motif (Harding et al., 2003; Su et al., 2009). A role for ATF4 in modulation of aging was suggested by evidence that lifespan extension in yeast, whether caused by 6OS ribosome mutations, lower TOR function, or nutrient deprivation, depended on the yeast homologue, Gcn4 (Steffen et al., 2008).

To see whether ATF4 might contribute to extended longevity in Snell dwarf mice, we evaluated ATF4 status in fibroblasts and liver of young adult Snell dwarf mice. In Snell dwarf fibroblasts, we noted augmented ATF4 responses to amino acid withdrawal, cadmium, H2O2, and the ER stress agent tunicamycin, with parallel increases in ATF4 itself as well as in multiple ATF4 target genes (Li & Miller, 2014). Liver of Snell dwarf mice also showed elevation of ATF4 protein, and of mRNA and protein for multiple ATF4 target genes. Parallel findings were also noted in fibroblasts and liver of PAPP-A KO mice, in which lifespan extension (Conover & Bale, 2007) is thought to reflect diminished IGF-1 availability secondary to higher tissue levels of IGF-1 binding proteins. Thus, both of these long-lived mice, with alterations in GH and/or IGF-1 action, showed augmented ATF4 levels in liver and heightened ATF4 responsiveness in skin-derived fibroblasts subjected to oxidant or ER stress.

To see whether elevated ATF4 function might be a feature shared by genetic and nongenetic models of slow aging in mice, we have now evaluated ATF4, ATF4-controlled proteins, and ATF4-controlled mRNA levels in liver of five kinds of mice known to have increased maximal longevity caused by nongenetic interventions: mice treated with acarbose (Harrison et al., 2014) or rapamycin (Harrison et al., 2009; Miller et al., 2011), mice subjected to caloric restriction (Flurkey et al., 2010) or methionine restriction (Miller et al., 2005; Sun et al., 2009), and mice kept in crowded litters from birth to weaning (Sun et al., 2009).

Acarbose (Aca) inhibits α-glucosidases in the intestine, thereby slowing the breakdown of complex polysaccharides to sugars and thus blunting the spike in blood glucose levels that follows a meal. It is an FDA-approved agent for treatment of human diabetes. Food intake is typically increased in Aca-treated mice (Yamamoto & Otsuki, 2006). The NIA Interventions Testing Program (ITP) has found (Harrison et al., 2014) that Aca increases median lifespan in genetically heterogeneous UM-HET3 mice by 22% in males (P < 0.0001) and by 5% in females (P = 0.01). Body weight was reduced, at 12 months of age, by 14% in males and by 22% in females, suggesting that the sexual dimorphism in longevity response does not parallel effects on body weight. Aca lowers fasting insulin levels in males but not in females, lowers IGF-1 levels in both sexes, and leads to a significant increase in blood levels of FGF-21, a mediator that declines dramatically in mice on a calorie-restricted (CR) diet (Harrison et al., 2014). Maximum lifespan, or more specifically the likelihood of survival to the 90th percentile age of the joint survival distribution (Wang et al., 2004), is increased in both males and females (P < 0.0001).

Rapamycin, an inhibitor of mTOR kinase, increases lifespan of both male and female UM-HET3 mice whether started at 9 or at 20 months of age (Harrison et al., 2009; Miller et al., 2011, 2014). At a dose of 42 parts per million (ppm), rapamycin leads to an increase of 23% in male lifespan and 26% in female lifespan (Miller et al., 2014). The stronger percentage response in females may reflect higher blood levels in females, compared with males consuming the same proportion of rapamycin in food (Miller et al., 2014). Rapamycin-treated mice also show retardation of age-related deficits in liver, tendon, heart, endometrium, and adrenal tissues, and delay in the age-sensitive decline in spontaneous activity (Wilkinson et al., 2012). Some age-sensitive physiological endpoints show improvement shortly after rapamycin treatment, some show beneficial effects only at older ages, and some do not show any improvement in an analysis of male C57BL/6 mice (Neff et al., 2013). Rapamycin also retards or reverses age-related declines in bone marrow production of B lymphocytes (Chen et al., 2009), age-dependent deficits in pulmonary responses to bacterial infection (Hinojosa et al., 2012), and development of both pathologic changes and behavioral deficits in a mouse model of Alzheimer’s disease (Caccamo et al., 2010). Rapamycin impairs glucose handling in multiple ways, with deficits in insulin production and response contributing in ways that depend on dose and time of exposure.

Caloric restriction (CR) is the best-studied method for slowing aging and extending lifespan in rodents, with beneficial effects on dozens of strains of rats and mice (Weindruch & Walford, 1988), including the UM-HET3 mice used in this study and the ITP protocol (Flurkey et al., 2010). Because CR diets diminish mTOR function, it has been suggested that inhibition of mTOR may be a shared feature of the anti-aging
pathways stimulated by CR and rapamycin (Johnson et al., 2013), but UM-HET3 mice exposed to rapamycin or to CR diets differ in many ways, including effects on fasting insulin and glucose levels, alteration in serum FGF-21 levels (depressed by CR diet and increased by rapamycin), and expression of hepatic genes involved in metabolism of xenobiotics (Miller et al., 2014).

Diets with reduced levels of methionine (Meth-R diets) lead to lifespan extension of 40% or more in rats and extend lifespan of multiple strains of rats despite an increase in food consumed per gram of body mass (Zimmerman et al., 2003). Meth-R diet also increases maximum lifespan in CB6F1 mice whether initiated at 6 weeks or at 12 months of age and retards development of age-related declines in T cell subset distribution and eye lens transparency (Miller et al., 2005; Sun et al., 2009). Although Meth-R mice weigh less than controls and have lower levels of IGF-1, insulin, and glucose (Miller et al., 2005), there are many physiological differences between the CR and Meth-R systems. There is, for example, little overlap in patterns of changed hepatic gene expression, and Meth-R mice do not show the increase in p38 and pERK kinase activation and decline in mTOR function seen in liver of CR mice (Sun et al., 2009).

In the crowded litter (CL) model, mice are foster nursed in groups of 12 (‘CL12’) pups per mother and compared with controls suckled in groups of 8 per mother (CL8 controls). Pups are weaned at 3 weeks of age and given unrestricted access to food thereafter. CL12 mice live 18% longer than controls (P = 0.0007) and show a significant increase in maximum lifespan (Sun et al., 2009). Although the intervention is thus limited to the first three weeks of life, patterns of liver gene expression remain distinct from controls at both 12 and 22 months of age (Steinaugh et al., 2012), and CL12 and CL15 mice show alterations in glucose tolerance and leptin sensitivity that last at least to 22 months (Sadagurski et al., 2014).

Results

Increased expression of ATF4, ATF3, and CHOP proteins in liver of five kinds of slow-aging mice

Both male and female mice exposed to acarbose from 4 to 12 months of age had significant elevations of ATF4 protein in liver (Fig. 1A). ATF3 and CHOP proteins, which are known to be modulated by ATF4 function, were also higher in acarbose-treated male and female mice (Fig. 1B,C). Figure 2A and B show representative immunoblots for these Aca-treated mice and controls. Similarly, ATF4, ATF3, and CHOP protein levels were elevated in male and female rapamycin-treated mice (age 22 months; Figure 1DEF; see Figure 2CD for representative immunoblot images). Significant increases in all three proteins were also seen in male mice from a group of CR mice (calorie restricted from 1.5 to 12 months; Figs 1G–I and 2E), and in 18-month-old male mice from a methionine-restricted cohort (Figs 1J–L and 2F). Lastly, female mice from a CL cohort, evaluated at 6 months, were found to have higher levels of all three proteins, with a significant effect (P < 0.05) seen for ATF4 and CHOP but not for ATF3 (Figs 1M–O and 2G).

Increased transcription of ATF4 target genes in five slow-aging mouse models

ATF4 regulates transcription of many genes, including (in liver) ATF3 (activating transcription factor 3), CHOP (CCAT/enhancer-binding protein homologous protein, also called GADD15), and ASNS (asparagine synthetase). Figure 3 shows hepatic mRNA levels for these three genes in each of the five varieties of slow-aging mice. When both sexes were available for study (acarbose, rapamycin, and CR), a two-factor analysis of variation was used to test for the overall effect of the intervention on the mRNA and to evaluate the interaction term as an indication of possible sex effects in responses to the intervention. The top line of Fig. 3 (panels ABC) shows that acarbose leads to a significant increase in ATF3, CHOP, and ASNS mRNA in liver of both male and female mice. The ‘Rx’ shown in each panel, derived from the two-factor ANOVA, indicates that the overall effect of acarbose is statistically significant, and the ‘Int’ designation, for ATF3 and ASNS, indicates that the two sexes had significantly different responses: ATF3 response was higher in females, and ASNS response was higher in males.

Figure 3 also shows significant overall effects of rapamycin and CR diet on each of the three ATF4-modulated mRNAs. The effects are significant for each sex considered independently, except for ASNS in female mice of the rapamycin cohort; the ANOVA showed that male and female responses to ASNS to rapamycin were indeed significantly different. Male mice treated with a methionine-restricted diet (Fig. 3, panels JKL) showed significant increases in each of the three mRNAs (females were not available for analysis). Methionine restriction produced a very large increase, over 400-fold, in ASNS mRNA, suggesting that this mRNA may be regulated by amino acid restriction in ways that do not reflect merely altered ATF4 function. For the CL intervention, only females were available for analysis, and these showed a significant increase in each of the three mRNA tested.

As a control, we examined expression of a panel of liver genes whose expression level is not thought to be related to ATF4 action, including Bnp, ATG12, NRF2, PEPCK1, Sirt1, and FasL, in each of the five varieties of slow-aging mice, and found no significant differences between treated and control mice (data not shown) for any of these mRNAs.

We also measured ATF4 mRNA in liver of each of the five varieties of slow-aging mice. Acarbose treatment led to a significant increase in ATF4 mRNA in males and females. Rapamycin treatment led to an increase in ATF4 mRNA in both sexes, significant in males. Methionine-restricted males showed a significant increase (females were not available for study). CR diet led to an increase in ATF4 mRNA in both sexes, but the effect was not statistically significant. Female CL mice showed a significant increase in ATF4 mRNA (males were not available for study). Thus, ATF4 mRNA levels in liver were elevated in all eight comparisons, with significant effects in 5 of the 8. The data are thus consistent with the protein measurements shown in Fig. 1.

To see whether ATF4-regulated mRNAs were modulated in other tissues, we measured the same three mRNA species in kidney and heart of mice exposed to acarbose, rapamycin, or CR diet, using six males and six females of each treated and age-matched control group (data not shown). All three mRNAs were significantly increased in kidney of male and female CR mice, and ATF3 mRNA increased significantly in kidney of rapamycin-treated males and females. For heart, we saw significant increases in ATF3 in male and female CR mice and of ASNS in male and female rapamycin-treated mice. We did not see any significant change in heart or kidney of acarbose-treated mice. Thus, our data show that ATF4 elevation is characteristic of liver in all five of the tested slow-aging mice (plus Snell and PAPP-A KO mice, reported previously), but effects of acarbose, rapamycin, and CR on kidney and heart are not as consistent as in liver.

Discussion

The availability of multiple methods to extend mouse maximal lifespan – genetic, dietary, development, or drug-induced – provides an opportunity
Fig. 1 Quantitation of protein expression of ATF4 and ATF4 targets. Levels of ATF4 protein (left: A, D, G, J, and M), ATF3 protein (middle: B, E, H, K, and N) and CHOP protein (right: C, F, I, L, and O) in liver of mice exposed to acarbose (top row: A, B, and C), rapamycin (second row: D, E, and F), calorie restriction (third row: G, H, and I), methionine restriction (fourth row: J, K, and L), or crowded litter intervention (bottom row: M, N, and O). Bars show means and SEM for N = 6 mice per age/condition shown. Only male mice were available for testing in the CR and Meth-R condition, and only female mice in the CL condition. Asterisk (*) indicates a significant effect of the intervention, at P < 0.05, using Sidak’s post hoc test after a one-factor analysis of variation.
to test the hypothesis that augmented ATF4 action, necessary for multiple modes of lifespan extension in yeast (Steffen et al., 2008), is also characteristic of slow aging in mice. Data in this paper show that ATF4 levels, levels of proteins controlled by ATF4, and levels of three mRNAs regulated directly by ATF4 are elevated in liver of mice exposed to each of five interventions shown elsewhere to increase maximal longevity: the drugs acarbose and rapamycin, diets low in calories or methionine, or transient milk deprivation limited to the suckling period. Our previous work (Li & Miller, 2014) has shown similar increases in ATF4 protein and downstream indicators of ATF4 function in liver of Snell dwarf mice and PAPP-A KO mice, in response to acarbose that might contribute to the sex-specificity of the lifespan results.

Our approach does not provide definitive evidence that higher ATF4 function is either necessary or sufficient for the extended longevity and extended good health seen in these many kinds of slow-aging mice. The data do, however, provide strong grounds for testing such ideas in future studies. For example, elevation of ATF4 might be used as a screen, in cell lines or in genetically malleable invertebrate organisms, for drugs that might deserve testing for beneficial effects in mice. Evaluation of ATF4 pathways in multiple tissues might help to reveal tissues in which altered ATF4 action is typically associated with slow aging. Our data, in conjunction with the data on yeast which inspired our study (Steffen et al., 2008), provide justification for evaluation of ATF4 dependent pathways in multiple vertebrate and invertebrate organisms.
Transcription of ATF4 targets in liver of long-lived mice. Levels of mRNA for ATF3 (left), CHOP (middle), and ASNS protein (right) in liver of mice exposed to acarbose (top row: A, B, and C), rapamycin (second row: D, E, and F), calorie restriction (third row: G, H, and I), methionine restriction (fourth row: J, K, and L), or crowded litter intervention (bottom row: M, N, and O). Bars show means and SEM for N = 6 mice per age/condition shown. Only male mice were available for testing in the CR and Meth-R condition, and only female mice in the CL condition. Data were analyzed by two-factor ANOVA, with tests for effects of intervention, sex, and the [sex x intervention] interaction term. Panels with the ‘Rx’ indicator had a significant overall effect of the intervention, and those with the ‘Int’ indicator had a difference between males and females in the response to the intervention. Asterisk (*) indicates a significant effect of the intervention, at P < 0.05, using Sidak’s post hoc test after a one-factor analysis of variation.
et al., 2011). There is also evidence that ATF4 regulates obesity, energy expenditure, and glucose homeostasis, in that ATF4-null mice are lean and mildly hypoglycemic and resist obesity induced by aging or high energy diets (Yoshizawa et al., 2009; Wang et al., 2010; Miller et al., 2011). Effects on lipid metabolism are mediated through alterations of PGC1a in white and brown adipose tissue (Wang et al., 2013). Furthermore, ATF4-deficient mice show reductions in hypertriglyceridemia induced by high fructose diets (Zhu et al., 2013) and increased insulin sensitivity (Zhang et al., 2013), suggesting that ATF4 may act as a modulator of lipid metabolism in the liver.

The extent to which these diverse ATF4-modulated pathways might be involved in control of aging rate, longevity, and the development of neoplastic diseases, which are the most diverse cause of death in mice, is at this point a matter for speculation and new experimentation. Construction of mice in which ATF4, or one or more of its immediate effectors, has been elevated or interrupted in specific cell types, or in which ATF4 pathways can be altered at specific postnatal time points, will also help to clarify the potential role played by ATF4 in postponement of disease and debility in aging animals. Evaluation of polymorphisms that modulate ATF4 level, or expression of a suite of ATF4-regulated mRNA, might also contribute to understanding of the genetic basis of specific disease of aging, or the relationship of genotype to overall health and longevity. Studies of ATF4 and its mediators in cells derived from long-lived and short-lived species could also produce insights into the possible role of the ATF4 system in evolutionary adaptation to niches that support slower aging and extended lifespan.

Methods

Mice and treatments

Acarbose: UM-HET3 mice, bred as the progeny of CB6F1 mothers and C3D2F1 fathers, were exposed to mouse chow containing acarbose at 1000 parts per million (ppm, equivalent to milligrams of drug per kilogram of chow) at 4 months of age and euthanized at 12 months of age. Mice in this population were also used for evaluation of hormonal and metabolic endpoints reported in (Harrison et al., 2014). Rapamycin: UM-HET3 mice were given food containing encapsulated rapamycin (Harrison et al., 2009) at 14 ppm from age 9 months and euthanized at 22 months of age. Calorie-restricted UM-HET3 mice (CR) were restricted to 80% of ad libitum food consumption from 6 to 8 weeks of age and to 60% of ad libitum consumption from 10 weeks of age until sacrifice at 12 months; metabolic and gene expression data from these mice were reported in (Miller et al., 2014). Mice fed with control mouse chow were used as controls for each of these three test groups; these control mice were born from the same breeding population, housed contemporaneously, and euthanized in parallel with the corresponding test group. CL12 mice were produced as described (Sun et al., 2009): experimental animals of the UM-HET3 stock were housed in groups of 12 newborns per foster mother from the first day of life until weaning at age 3 weeks, and then given free access to food after weaning. Controls for the CL12 mice were foster nursed in groups of 8 newborns per mother, and given free access to food after weaning. CL12 and their controls were euthanized at 6 months of age. Except for the acarbose group, mice were euthanized between 7 and 10 am and were not fasted prior to euthanasia. Mice in the acarbose group, and their controls, were fasted for four hr prior to sacrifice and killed between noon and 1 pm. All animal procedures were as approved by the University Committee on Use and Care of Animals at the University of Michigan. The colony is evaluated for specific pathogen status every 3 months, and all such tests were negative during the period of this study.

Methods for sample preparation, immunoblotting, and quantitative real-time PCR are as described in (Li & Miller, 2014).

Statistics and graphics

Bar graphics show mean values for N = 6 individual mice of each sex and treatment group, and error bars indicate standard errors of the mean. Protein levels are expressed in each panel relative to the mean level of protein or mRNA in control mice of the same sex. mRNA values are expressed relative to the levels in control male mice.

Acknowledgments

We thank Sabrina Van Roekel and Amanda Keedle for animal husbandry assistance.

Funding

This work was supported by NIH grants AG013283, AG024824, and AG019899 and by a Senior Scholar award from the Ellison Medical Foundation.

Conflict of interest

None declared.

References

Al Sarraj J, Vinson C, Thiel G (2005) Regulation of asparagine synthetase gene transcription by the basic region leucine zipper transcription factors ATF5 and CHOP. Biol. Chem. 386, 873–879.

Caccamo A, Majumder S, Richardson A, Strong R, Oddo S (2010) Molecular interplay between mammalian target of rapamycin (mTOR), amyloid-beta, and Tau: effects on cognitive impairments. J. Biol. Chem. 285, 13107–13120.

Chen C, Liu Y, Liu Y, Zheng P (2009) mTOR regulation and therapeutic rejuvenation of aging hematopoietic stem cells. Sci. Signal. 2, ra75.

Conover CA, Bale LK (2007) Loss of pregnancy-associated plasma protein A extends lifespan in mice. Aging Cell 6, 727–729.

Costa-Mattioli M, Gobert D, Stern E, Gamache K, Colina R, Cuello C, Sossin W, Kaufman R, Pelletier J, Rosenblum K, Krijevic K, Lacaille JC, Nader K, Sonenberg N (2007) eIF2alpha phosphorylation bidirectionally regulates the switch from short- to long-term synaptic plasticity and memory. Cell 129, 195–206.

Ebert SM, Monteys AM, Fox DK, Bongers KS, Shields BE, Malmberg SE, Davidson BL, Suneja M, Adams CM (2010) The transcription factor ATF4 promotes skeletal muscle fiber atrophy during fasting. Mol. Endo. 24, 790–799.

Flurkey K, Astle CM, Harrison DE (2010) Life extension by diet restriction and N-acetyl-L-cysteine in genetically heterogeneous mice. J. Gerontol. A Biol. Sci. Med. Sci. 65, 1275–1284.

Harding HP, Zhang Y, Zeng H, Novaoa I, Lu PD, Caflon M, Sadri N, Yun C, Popko B, Paules R, Stojdl DF, Bell JC, Hettmann T, Leiden JM, Ron D (2003) An integrated stress response regulates amino acid metabolism and resistance to oxidative stress. Mol. Cell 11, 619–633.

Harrison DE, Strong R, Sharp ZD, Nelson JF, Astle CM, Flurkey K, Nadon NL, Wilkinson JE, Frenkel K, Carter CS, Pahor M, Javors MA, Fernandez E, Miller RA (2009) Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. Nature 460, 392–395.

Harrison DE, Strong R, Allison DB, Ames BN, Astle CM, Atamna H, Fernandez E, Flurkey K, Javors MA, Nadon NL, Nelson JF, Fletcher S, Simpkins JW, Smith D, Wilkinson JE, Miller RA (2014) Acarbose, 17-alpha-estradiol, and nordihydroguaiaretic acid extend mouse lifespan preferentially in males. Aging Cell 13, 273–282.

Hinojosa CA, Mgbenema V, Van Roekel S, Austad SN, Miller RA, Bose S, Orihuela CJ (2012) Enteric-delivered rapamycin enhances resistance of aged mice to...
pneumococcal pneumonia through reduced cellular senescence. Exp. Gerontol. 47, 956–965.

Johnson SC, Rabinovitch PS, Kaebelerlein M (2013) mTOR is a key modulator of ageing and age-related disease. Nature 493, 338–345.

Li W, Miller RA (2014) Elevated ATF4 function in fibroblasts and liver of slow-aging mutant mice. J. Gerontol. A Biol. Sci. Med. Sci. doi:10.1093/gerona/glu040.

Masuoka HC, Townes TM (2002) Targeted disruption of the activating transcription factor 4 gene results in severe fetal anemia in mice. Blood 99, 736–745.

Miller RA, Buehner G, Chang Y, Harper JM, Sigler R, Smith-Wheelock M (2005) Methionine-deficient diet extends mouse lifespan, slows immune and lens aging, alters glucose, T4, IGF-I and insulin levels, and increases hepatocyte MIF levels and stress resistance. Aging Cell 4, 119–125.

Miller RA, Harrison DE, Astle CM, Baur JA, Boyd AR, de CR, Fernandez E, Flurkey K, Han M, Javors MA, Li X, Masuoka HC, Townes TM (2002) Targeted disruption of the activating transcription factor 4 gene results in severe fetal anemia in mice. Blood 99, 736–745.

Steinbaugh MJ, Sun LY, Bartke A, Miller RA (2012) Activation of genes involved in xenobiotic metabolism is a shared signature of mouse models with extended lifespan. Am. J. Physiol. Endocrinol. Metab. 303, E488–E495.

Su N, Thiaville MM, Aikawa K, Gjymishka A, Brant JO, Yang TP, Killberg MS (2009) Protein or amino acid deprivation differentially regulates the hepatic forkhead box protein A (FOXO) genes through an activating transcription factor-4-independent pathway. Hepatology 50, 282–290.

Sugiyaama RH, Arfin SM, Harris M (1983) Properties of asparagine synthetase in asparagine-independent variants of jensen rat sarcoma-cells induced by 5-aza- cytidine. Mol. Cell. Biol. 3, 1937–1942.

Sun L, Sadigki Akha AA, Miller RA, Harper JM (2009) Life-span extension in mice by preweaning food restriction and by methionine restriction in middle age. J. Gerontol. A Biol. Sci. Med. Sci. 64, 711–722.

Tanaka T, Tsujimura T, Takeda K, Sugihara A, Maekawa A, Terada N, Yoshida N, Akira S (1998) Targeted disruption of ATF4 discloses its essential role in the formation of eye lens fibres. Genes Cells 3, 801–810.

Wang C, Li Q, Redden DT, Weindruch R, Allison DB (2004) Statistical methods for testing effects on “maximum lifespan”. Mech. Aging Dev. 125, 629–632.

Wang CX, Huang ZY, Du Y, Cheng Y, Chen SH, Guo FF (2010) ATF4 regulates lipid metabolism and thermogenesis. Cell Res. 20, 174–184.

Wang XQ, Wang GB, Kunte M, Shinde V, Gorbatyuk M (2013) Modulation of Angiogenesis by Genetic Manipulation of ATF4 in a Mouse Modulation of Angiogenesis by Genetic Manipulation. Invest. Ophthalmol. Vis. Sci. 54, 5995–6002.

Weindruch R, Walford RL (1988) The Retardation of Aging and Disease by Dietary Restriction. Springfield, IL: Charles C Thomas.

Wilkinson JE, Burmeister L, Brooks SV, Chan CC, Friedline S, Harrison DE, Hejtmancik JF, Nadon N, Strong R, Wood LD, Woodward MA, Miller RA (2012) Rapamycin slows aging in mice. Aging Cell 11, 675–682.

Yamamoto M, Matsuoka K (2006) Effect of inhibition of alpha-glucosidase on age-related glucose intolerance and pancreatic atrophy in rats. Metabolism 55, 533–540.

Yoshizawa T, Hinoi E, Jung DY, Kajimura D, Ferron M, Seo J, Graff JM, Kim JK, Karsenty G (2009) The transcription factor ATF4 regulates glucose metabolism in mice through its expression in osteoblasts. J. Clin. Invest. 123, 3272–3291.

Ren Y, Roy S, Ding Y, Iqbal J, Broome JD (2014) Methylation of the asparagine-dependent variants of jensen rat sarcoma-cells induced by 5-aza- cytidine. Mol. Cell. Biol. 3, 1937–1942.