

Integration of Diverse Inputs in the Regulation of *Caenorhabditis elegans* DAF-16/FOXO

Jessica N. Landis and Coleen T. Murphy*

In a remarkably conserved insulin signaling pathway that is well-known for its regulation of longevity in worms, flies, and mammals, the major *C. elegans* effector of this pathway, DAF-16/FOXO, also modulates many other physiological processes. This raises the question of how DAF-16/FOXO chooses the correct targets to achieve the appropriate response in a particular context. Here, we review current knowledge of tissue-specificity and interacting partners that modulate DAF-16/FOXO functional output. *Developmental Dynamics* 239:1405–1412, 2010. © 2010 Wiley-Liss, Inc.

Key words: DAF-16; FOXO; DAF-2; insulin; aging; longevity; *C. elegans*

Accepted 5 January 2010

INTRODUCTION

Over the past two decades, there has been significant growth in our understanding of the genetics underlying aging, catalyzed by the discovery that single-gene mutations can dramatically extend lifespan in the model organism *Caenorhabditis elegans* (Friedman and Johnson, 1988; Kenyon et al., 1993). Importantly, one such mutation discovered to double the worm's lifespan is the *C. elegans* insulin receptor (*daf-2*) (Kenyon et al., 1993); this effect is completely dependent on the gene *daf-16* (Kenyon et al., 1993). The insulin/IGF-1 signaling (IIS) pathway's components are highly conserved in worms, flies, and mammals, and the FOXO transcription factor, DAF-16, is the major downstream effector of DAF-2, *C. elegans*' sole insulin/IGF-1-like receptor (Tatar et al., 2003).

In addition to its well-known regulation of longevity, DAF-16/FOXO is crucial for many important processes, including development, stress resist-

ance, thermotolerance, pathogen resistance, metabolism, and autophagy (Vowels and Thomas, 1992; Baugh and Sternberg, 2006; Murakami and Johnson, 1996; Henderson and Johnson, 2001; Garsin et al., 2003; Ogg et al., 1997; Kimura et al., 1997; Jia et al., 2009). The transcriptional targets of DAF-16, which presumably execute its many roles, include stress-response, antimicrobial, and metabolic genes, as well as many genes of unknown function (Murphy et al., 2003; McElwee et al., 2003; Lee et al., 2003; Oh et al., 2006), and many of the genes most highly regulated by DAF-16 influence aging (Murphy et al., 2003). Thus, the convergence of IIS signaling on these diverse outputs raises the question of how DAF-16 specificity is achieved. Many of the recent breakthroughs in the field involve the identification of new DAF-16/FOXO co-factors and their effects on DAF-16/FOXO functional output. Here we discuss developments in the area of DAF-16/FOXO co-factors that

may help DAF-16 regulate these diverse functions.

INSULIN SIGNALING REGULATION OF DAF-16

daf-2 was originally identified for its mutant's dauer constitutive (Daf-C) phenotype, forming the alternative larval state inappropriately under growth-promoting conditions, while *daf-16* was identified in a Daf-D (dauer defective) mutant that suppressed the *daf-2* mutant's dauer formation (Vowels and Thomas, 1992; Gottlieb and Ruvkun, 1994). In conditions suitable for growth and reproduction, activation of DAF-2 by binding of an agonist insulin-like ligand initiates a phosphorylation cascade that ultimately inhibits DAF-16 activity. Remarkably, there are 40 insulin-like molecules encoded in the *C. elegans* genome, some of which may function as DAF-2 agonists, and others as antagonists (Pierce et al., 2001; Li et al., 2003; Kawano et al.,

Department of Molecular Biology, Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, New Jersey
*Correspondence to: Coleen T. Murphy, Department of Molecular Biology, Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ 08544. E-mail: ctmurphy@princeton.edu

DOI 10.1002/dvdy.22244

Published online 5 February 2010 in Wiley InterScience (www.interscience.wiley.com).

2000; Murphy et al., 2007). Activated DAF-2 phosphorylates the phosphoinositide 3-kinase, AGE-1, generating PIP3, which, in turn, recruits the kinases AKT-1, AKT-2, SGK-1, and PDK-1 to the plasma membrane, where PDK-1 phosphorylates AKT and SGK-1 (Hertweck et al., 2004; Paradis et al., 1999). The AKT-1/AKT-2/SGK-1 complex phosphorylates the forkhead transcription factor DAF-16 (Paradis and Ruvkun, 1998; Hertweck et al., 2004), sequestering it in the cytoplasm (Ogg et al., 1997; Lin et al., 1997), thus preventing DAF-16 from activating or repressing transcription of its target genes in the nucleus (Fig. 1). Inhibitors of this cascade include DAF-18, the phosphoinositide 3-phosphatase PTEN, which antagonizes AGE-1 by dephosphorylating PIP3 (Ogg and Ruvkun, 1998), and PPTR-1, a regulatory subunit of PP2A that dephosphorylates AKT-1 (Padmanabhan et al., 2009).

DAF-16 May Act in Specific Tissues to Execute Different Outputs

As *C. elegans* is a multi-tissued organism, it is important to establish where DAF-16 may act to execute its diverse functions. *daf-2* acts cell-non-autonomously to specify life span and formation of the stress-resistant dauer developmental stage (Apfeld and Kenyon, 1998), although the sites of action of DAF-2 and DAF-16 remain controversial. Restoration of DAF-2 and AGE-1 expression in neurons, but not the intestine, is sufficient to shorten the life span of long-lived *daf-2* and *age-1* mutant animals, respectively (Wolkow et al., 2000). However, subsequent results showed that AGE-1 in the intestine can shorten the life span of an *age-1* mutant (Iser et al., 2007). The reason for this discrepancy is unclear, but may result from differences in transgenes or expression levels.

At the level of DAF-16 activity, Libina et al. (2003) showed that DAF-16 activity in the intestine partially restores the life span extension (60%) of *daf-16;daf-2* mutants, suggesting that the intestine is an important site of DAF-16's role in longevity, whereas neuronal DAF-16 effects a much lesser life span extension. By con-

trast, DAF-16 expressed in the neurons was sufficient to promote dauer formation, whereas DAF-16 in the intestine had very little effect on dauer formation (Libina et al., 2003), which indicates that some DAF-16 functionality may arise from its activity in specific tissues. Iser et al. (2007) showed that tissue-restricted *daf-16* expression in the intestine or neurons of a *daf-16;age-1* mutant does not restore life span as strongly as does *daf-16* expressed in multiple tissues, perhaps suggesting that DAF-16 may act in multiple tissues in a graded fashion to specify longevity. Finally, tissue-restricted *daf-16* expression in the hypodermis resulted in embryonic or larval lethality (Libina et al., 2003), suggesting that expression of *daf-16* in the hypodermis may be lethal unless accompanied by expression elsewhere. This result underscores the importance of appropriate DAF-16 regulation for the animal.

Such tissue-specific insulin receptor and FOXO activity is unlikely to be restricted to *C. elegans*. For example, in mice, loss of the insulin receptor in adipose (fat) tissue (the "FIRKO mouse") extends life span (Blüher et al., 2003). However, loss of insulin signaling is not generally beneficial, as its loss in the liver causes diabetes (Michael et al., 2000). In flies, overexpression of dFOXO in the adipose tissue extends life span (Giannakou et al., 2004). The fact that both FOXO and insulin/IGF-1 receptors have been identified as genes linked to extreme longevity in recent human centenarian studies (Wilcox et al., 2008; Flachsbarth et al., 2009; Suh et al., 2008) suggests that discovering the downstream, tissue-specific FOXO-regulated targets, as in model organisms, will be important for understanding FOXO's role in human longevity and aging.

DAF-16 May Receive Signals From the Gonad

The *C. elegans* reproductive system regulates longevity signaling from the somatic gonad and the germ line, two tissues with opposing effects on longevity. Longevity is extended by germ line removal, an effect that is dependent on *daf-16*, but this extension is inhibited when both the somatic

gonad and germ line are removed (Hsin and Kenyon, 1999). Removing the germ cells results in nuclear accumulation of DAF-16 in intestinal cells (Lin et al., 2001), and DAF-16 activity only in the intestine is completely sufficient for this germ line-mediated longevity (Libina et al., 2003). This effect requires the activity of KRI-1, an intestinally expressed ankyrin-repeat protein orthologous to the human disease gene KRIT1/CCM1 (Berman and Kenyon, 2006). The nuclear hormone receptor DAF-12 (Antebi et al., 2000) and the cytochrome P450 DAF-9 (which is postulated to help synthesize a lipophilic ligand for DAF-12; Motola et al., 2006) are also required for nuclear localization of DAF-16 (Berman and Kenyon, 2006). These results suggest that the germline communicates with DAF-16 in the intestine via endocrine signaling, and that the response to this signaling requires KRI-1. Similar regulation of germline-mediated longevity has been recently uncovered in flies (Flatt et al., 2008).

Thus, DAF-16 carries out specific roles both directly in and in response to signaling from different tissues, resulting in specific biological outputs, and such activity is likely at least partially conserved in higher organisms.

DAF-16 CO-REGULATORS AND CO-FACTORS

While specific DAF-16 functions arise from activity in different tissues, DAF-16 is not differentially excluded from specific tissues during different life stages (Henderson and Johnson, 2001; Ogg et al., 1997). Thus, the question arises, how does DAF-16 choose its tissue-specific targets to effect these different outputs? Several lines of evidence suggest that DAF-16 does not act alone, but rather requires other molecules for its activity. First, simply increasing the amount of DAF-16 is not sufficient to confer its effect on longevity, as overexpression of DAF-16 in wild-type worms only increases life span slightly (Henderson and Johnson, 2001). Second, although nuclear localization of DAF-16 is necessary for target activation, nuclear localization is not sufficient to increase lifespan (Lin et al., 2001).

Third, the canonical DAF-16 Binding Element (DBE) (Furuyama et al., 2000) is present in the 5-kb upstream of 78% of *C. elegans* genes (Kenyon and Murphy, 2006), yet only a small number of these genes are DAF-16 targets in young adulthood (Murphy et al., 2003). Thus, the presence of the DBE is not sufficient to predict DAF-16-induced expression, which suggests that there may be other factors to help DAF-16 to discriminate among potential targets. In this section, we describe putative DAF-16 co-factors and co-regulators (Fig. 2), and discuss

how they may contribute to DAF-16's multiple roles.

JNK-1

c-Jun N-terminal kinase (JNK), a stress-activated MAPK family member, is a positive regulator of FOXO activity in worms and mammals. Overexpression of the mammalian JNK ortholog *jnk-1* extends *C. elegans*' lifespan and increases resistance to heat and oxidative stress in a *daf-16*-dependent manner (Oh et al., 2005). JNK directly phosphorylates DAF-16 in vitro, and genetic and biochemical data suggest that *jnk-1* acts in parallel to the insulin signaling pathway to regulate DAF-16, but in an opposite manner; that is, phosphorylation of DAF-16 by JNK promotes nuclear localization of DAF-16 and its subsequent activation (Fig. 2). Similarly, JNK phosphorylates the mammalian homolog FOXO4 in vivo, resulting in FOXO4 translocation from the cytoplasm to the nucleus and transcriptional activation (Essers et al., 2004).

residue in the DNA-binding domain, triggering dissociation of FOXO3 and 14-3-3 proteins, accumulation of FOXO3 in the nucleus, and neuronal cell death (Lehtinen et al., 2006). Similarly, MST1 phosphorylates *C. elegans* DAF-16 at the corresponding conserved site. Interestingly, while mammalian MST1-FOXO activation promotes neuronal cell death, the worm homolog CST-1 promotes longevity. Like JNK-1, overexpression of CST-1 extends lifespan in a *daf-16*-dependent manner, and further extends the longevity of *daf-2* knockdown animals, suggesting that CST-1 may also act in parallel to the insulin signaling pathway to regulate DAF-16 activity (Lehtinen et al., 2006).

SIR-2

First discovered in yeast for their roles in epigenetic silencing (Rine and Herskowitz, 1987), sirtuins are NAD⁺-dependent deacetylases whose overexpression increases longevity in yeast, worms, and flies (Kaerberlein et al., 1999; Tissenbaum and Guarente, 2001; Rogina and Helfand, 2004). Similar to overexpression, chemical activators of sirtuins, including the well-known compound resveratrol, extend life span in worms and flies (Wood et al., 2004). Expression of extra copies of *sir-2.1*, one of the four *C. elegans* Sir2 homologs, increases life span and resistance to

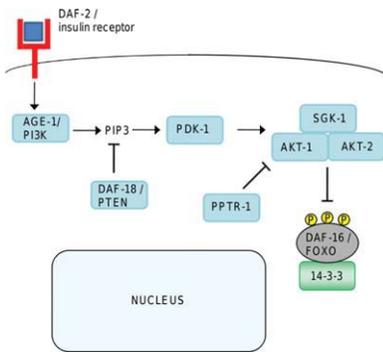


Fig. 1. Insulin signaling regulation of DAF-16/FOXO. Activated DAF-2 initiates a phosphorylation cascade that ultimately phosphorylates DAF-16, thereby excluding it from the nucleus and inhibiting its activity. As noted in the text, it remains unclear whether DAF-16 interacts with both 14-3-3 proteins FTT-2 and PAR-5, so here we have labeled its interacting partner "14-3-3."

CST-1

Like JNK, the conserved mammalian Ste20-like kinase, MST1, and its *C. elegans* homolog, CST-1, activate FOXO in response to oxidative stress. In mammalian neuronal cells, MST1 phosphorylates FOXO3 at a conserved

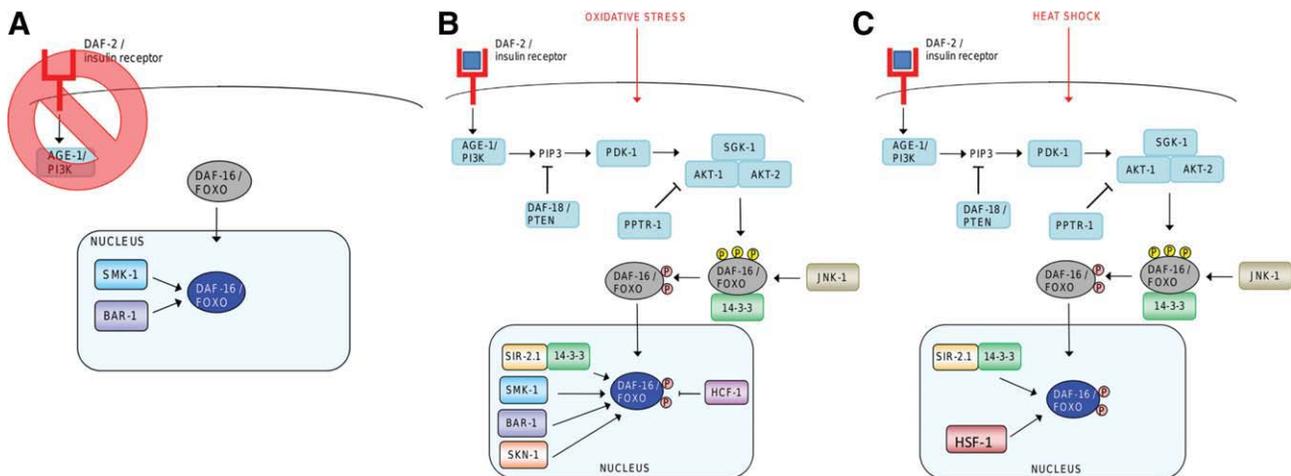


Fig. 2. DAF-16 interacts with specific proteins under different stimuli. **A:** Reduced insulin signaling relieves the phosphorylation of DAF-16, allowing it to enter the nucleus and interact with SMK-1 and BAR-1. **B:** In response to oxidative stress, JNK-1 activates DAF-16 by phosphorylation. (Note that the phosphatase that may remove the inhibitory AKT/SGK phosphates to allow nuclear translocation is unknown, but the serine/threonine phosphatase PP2A dephosphorylates mammalian FOXO1) (Yan et al., 2008). Activated DAF-16 accumulates in the nucleus, where it interacts with SIR-2.1/14-3-3, SMK-1, BAR-1, SKN-1, and HCF-1. **C:** Heat shock causes JNK-1 to phosphorylate and activate DAF-16, which, in turn, enters the nucleus and interacts with SIR-2.1/14-3-3 and HSF-1.

thermal and oxidative stress, in a *daf-16*/FOXO-dependent manner (Tissenbaum and Guarente, 2001).

The mammalian ortholog of *sir-2*, SIRT1, deacetylates FOXO3a and FOXO4 (Brunet et al., 2004; van der Horst et al., 2004). As *C. elegans* DAF-16 shares several lysines that have been identified as acetylated residues in mammalian FOXO3a (Brunet et al., 2004), it is likely that a similar interaction exists between *C. elegans* SIR-2.1 and DAF-16. Interestingly, like the insulin-mediated phosphorylation and nuclear localization of DAF-16, the presence of FOXO3 in the nucleus alone, by mutation of the three Akt phosphorylation sites, is not sufficient to promote interaction between SIRT1 and FOXO3a in the absence of stress signals (Brunet et al., 2004). This suggests that stress-inducible FOXO3a acetylation and phosphorylation events may promote this interaction, and further demonstrates the complexity of FOXO control.

Notably, SIRT1 has a dual effect on FOXO3a function: SIRT1 increases FOXO3a's induction of cell-cycle arrest and resistance to oxidative stress, but inhibits FOXO3a's induction of apoptosis (Brunet et al., 2004). It will be interesting to explore whether *C. elegans* SIR-2.1 can also differentially modulate aspects of DAF-16 function in a context-dependent manner.

14-3-3

The 14-3-3 proteins are members of a highly conserved family present in all eukaryotes examined (van Hemert et al., 2001). They can act as localization anchors or as adaptor proteins with scaffold activity that facilitates protein-protein interactions (van Hemert et al., 2001). The *C. elegans* genome encodes two 14-3-3 genes, *par-5* (also known as *ftt-1*) and *ftt-2* (Wang and Shakes, 1997). Demonstrating a role for 14-3-3 proteins in the regulation of DAF-16, reduced expression of *ftt-2* enhances dauer formation, and also promotes DAF-16 nuclear localization and activation of several target genes (Li et al., 2007). FTT-2 directly binds to DAF-16, and this association is reduced under *daf-2*(-) conditions, consistent with the model that in reduced insulin signal-

ing conditions, DAF-16 is dephosphorylated and dissociates from 14-3-3 in the cytoplasm. This regulation is conserved, as mammalian 14-3-3 ζ also binds and sequesters FOXO3a in the cytoplasm (Brunet et al., 1999).

Whether PAR-5 regulates DAF-16 remains unclear, due to the high sequence identity between *ftt-2* and *par-5* and resultant cross-recognition by *par-5* RNAi clones and antibodies. Berdichevsky et al. (2006) found that, like *ftt-2*, reducing *par-5* results in nuclear accumulation of DAF-16. Additionally, Li et al. (2007) noted that a low level of PAR-5 also co-immunoprecipitated with DAF-16; this could be due to the PAR-5 antibody cross-reacting with FTT-2, or, alternatively, PAR-5 may in fact interact with DAF-16. Interestingly, *ftt-2* RNAi (Li et al., 2007; Berdichevsky et al., 2006) and *par-5* RNAi (Berdichevsky et al., 2006) shorten the life span of wild-type worms, but neither affects the life span of *daf-2*(-) animals. The SIR-2 overexpression/DAF-16-dependent increases in life span and heat and oxidative stress resistance are dependent on *ftt-2*, and *par-5* may function in life span extension (Berdichevsky et al., 2006). Using immunoprecipitation/mass spectrometry and yeast two-hybrid assays, respectively, Wang et al. (2006) showed that SIR-2 interacts with PAR-5 and FTT-2, and in response to heat stress, but not low insulin-like signaling, SIR-2.1 and DAF-16 physically interact in the nucleus, dependent on *ftt-2* (Berdichevsky et al., 2006). 14-3-3 proteins bridge the interaction between SIR-2.1 and DAF-16 in response to stress; thus it is possible that, although there is more DAF-16 in the nucleus of *ftt-2*, and perhaps *par-5*, knockdown worms, this is countered by dissociation of the 14-3-3/SIR-2.1/DAF-16 complex and perhaps other protein complexes.

HSF-1

Heat-shock factor (HSF) induces transcription of chaperones and proteases in response to heat and other stressors. In *C. elegans*, reducing the activity of HSF-1 causes a rapid-tissue-aging phenotype and shortens life span (Garigan et al., 2002). *hsf-1* is

required for *daf-2* dauer formation, longevity, and pathogen resistance (Morley and Morimoto, 2004; Hsu et al., 2003; Singh and Aballay, 2006). Conversely, HSF-1 overexpression increases heat resistance and extends life span in a DAF-16-dependent manner (Hsu et al., 2003), suggesting that DAF-16 and HSF-1 act together to promote longevity. Indeed, HSF-1 and DAF-16 act together to induce small heat-shock protein genes, all of which have consensus DAF-16 and HSF-1 binding sites in upstream regions. Yet, HSF-1 inactivation does not prevent DAF-16 accumulation in the nuclei of *daf-2* mutants, or the expression of the stress-responsive DAF-16 target genes *sod-3* and *mtl-1* (Hsu et al., 2003). Likewise, the heat-inducibility of several HSF-1 targets is not diminished by loss of *daf-16*. Thus, HSF-1 and DAF-16 appear to function together to turn on a specific subset of genes.

Interestingly, while reduction of *hsf-1* does not affect nuclear localization of DAF-16 under reduced IIS conditions, RNAi knockdown of *hsf-1* and its target *Hsp70/hsp-1* delay nuclear export of DAF-16 during recovery from heat shock (Singh and Aballay, 2009), showing that HSF-1 may have a role in controlling DAF-16 activity. The mechanism of this regulatory interaction remains to be elucidated.

β -Catenin

Best known for mediating Wnt signaling by binding to members of the T-cell factor (TCF) family of transcription factors, the *C. elegans* β -catenin homolog, *bar-1*, also interacts with DAF-16. Loss of *bar-1* reduces the activity of DAF-16 in promoting dauer formation. Furthermore, like *daf-16* mutants, *bar-1* mutants are short lived and are more sensitive to oxidative stress (Essers et al., 2005). *bar-1* is required for oxidative stress-induced expression of the direct DAF-16 target *sod-3* and physically binds to DAF-16. Thus, these results demonstrate a direct role for β -catenin in regulating DAF-16/FOXO function.

This β -catenin interaction with FOXO is evolutionarily conserved in mammalian cells. As in *C. elegans*, mammalian β -catenin physically interacts with the homologs FOXO1

and FOXO3a, and binding between β -catenin and FOXO is enhanced in response to oxidative stress. β -catenin in mammalian cells increases the expression of FOXO activity reporters as well as an endogenous cell cycle target gene (Essers et al., 2005). Moreover, at the functional level, β -catenin enhances the ability of FOXO to inhibit cell-cycle progression. By contrast, β -catenin-TCF signaling has a role in cancer development. Thus, an exciting possibility is that FOXO could compete with TCF for β -catenin interaction, and thereby inhibit cell-cycle and tumor progression. In support of this model, binding between β -catenin and TCF is reduced by FOXO overexpression and oxidative stress (Hoogeboom et al., 2008).

SMK-1

The highly conserved SMEK (suppressor of MEK) nuclear factor was identified in *Dictyostelium discoideum* as a suppressor of *mek1* cell polarization and motility defects (Mendoza et al., 2005), and a role in longevity was discovered in *C. elegans* soon thereafter. Similar to *daf-16* mutation, reduced *smk-1* completely suppresses the extended longevity of a *daf-2* mutant and slightly shortens the life span of wild-type worms (Wolff et al., 2006). Also like *daf-16*, *smk-1* is required for the long life span of germ-line ablated animals. Moreover, SMK-1 is required for DAF-16-mediated innate immune, UV, and oxidative stress response, but not for thermotolerance. Corresponding to these physiological data, molecular data indicate that *smk-1* RNAi reduces expression of oxidative stress response (*sod-3*, *ctl-1*) and pathogen response (*lys-8*) genes, but does not reduce the expression of a heat-stress-inducible gene (*hsp-12.6*). Notably, SMK-1 is also required for the transcriptional repression of *daf-15/raptor* by DAF-16, suggesting that SMK-1 mediates transcriptional activator and repressor activities of DAF-16. Although it remains to be determined whether SMK-1 forms a complex with DAF-16, SMK-1 temporally and spatially co-localizes with DAF-16 in the nuclei of intestinal cells, head and tail neurons, and hypodermal cells (Wolff et al., 2006).

Taken together, these data suggest the possibility that SMK-1 is a nuclear co-regulator that acts specifically to modulate a subset of DAF-16's function.

HCF-1

Whereas the previously discussed putative co-factors represent positive regulators of DAF-16, the *C. elegans* homolog of host cell factor 1 (HCF-1) functions as a negative regulator of DAF-16 (Li et al., 2008). HCF-1 inactivation extends life span and may function in a pathway in parallel to IIS, as loss of *hcf-1* and loss of *daf-2/age-1* act synergistically to extend lifespan. While loss of HCF-1 increases resistance to oxidative and heavy metal stress, both effects that require DAF-16, it does not affect heat shock survival. Consistent with these uncoupled physiological outputs, HCF-1 regulates the DAF-16-mediated transcription of a subset of previously identified DAF-16 target genes. Loss of *hcf-1* leads to enrichment of DAF-16 on the promoters of several DAF-16-upregulated target genes, suggesting that in the absence of HCF-1, more DAF-16 is able to bind to the promoters of its targets. HCF-1 is ubiquitously expressed, is constitutively localized to nuclei, and physically associates with DAF-16, providing evidence that HCF-1 functions as a DAF-16 co-factor or co-regulator (Li et al., 2008).

SKN-1

The *C. elegans* transcription factor SKN-1/Nrf defends against oxidative stress by activating the conserved phase 2 detoxification system (An and Blackwell, 2003). Additionally, SKN-1 upregulates numerous other genes involved in detoxification, cellular repair, pathogen resistance, and others, and downregulates a set of genes that reduce stress resistance and life span (Oliveira et al., 2009). SKN-1 is expressed in the intestine, where it mediates the phase 2 stress response, and in the ASI neurons (An and Blackwell, 2003). Similar to the regulation of DAF-16 by insulin signaling, the IIS kinases phosphorylate SKN-1, and reduced IIS leads to constitutive SKN-1 accumulation in in-

testinal nuclei and target gene activation (Tullet et al., 2008). Furthermore, SKN-1 contributes to the longevity and stress-resistance phenotypes of reduced insulin signaling. These results raise the possibility that SKN-1 acts together with DAF-16 in regulating some processes and target genes. In support of this, DAF-16 contributes to the induction of a subset of SKN-1 targets under reduced insulin signaling conditions (Tullet et al., 2008). Further work will elucidate the sites and nature of SKN-1/DAF-16 interaction.

The DAF-16-Associated Element Binding Factor

An unbiased search for sequence motifs overrepresented in upstream regions of DAF-16 targets identified the in vitro-defined canonical DAF-16-binding element (DBE; TGTTTAC) (Furuyama et al., 2000), and a new GATA sequence, the DAF-16 Associated Element (DAE; CTTATCA) (Murphy et al., 2003). Both of these motifs are present in the promoters of up- and downregulated targets. Furthermore, the upstream regions of dauer-enriched, *age-1*-regulated genes, and age-modulated genes are also enriched for a GATA motif that is similar to that of the DAE ([T/C/G]GATAA[C/G][A/G]) (Budovskaya et al., 2008). Because the DAE differs significantly from the DBE, it is difficult to imagine how DAF-16 would also bind to the DAE; rather, a known transcription factor or currently unknown transcription factor may bind this motif and regulate these genes.

There are 14 GATA transcription factors in *C. elegans* (Budovskaya et al., 2008) that are candidate DAE-binding factors. Among these, RNAi knockdown of the *elt-3* GATA transcription factor suppresses the longevity of *daf-2* mutants (Budovskaya et al., 2008). Thus, *elt-3* functions to promote life span, making it an attractive candidate for the unknown DAE-binding factor. *elt-3* is expressed in hypodermal cells, the intestine, the pharyngeal-intestinal valve cells, and the intestinal-rectal valve cells, and shows a complex pattern of age-related change in these tissues.

Reduction of *elt-3* attenuates the expression of three age-regulated genes in the tissues where *elt-3* and the target is co-expressed, and mutation of the GATA DNA sites of these reporters reduces their expression. One of these genes, *sod-3*, is a known direct DAF-16 target (Honda and Honda, 1999) and expression of the other two increases in a reduced IIS condition. Thus, *elt-3* is a reasonable candidate DAE-binding factor, but further work must be done to determine whether *elt-3* directly binds these GATA sites, for example, by using ChIP-seq to compare ELT-3 targets to direct DAF-16 targets (Oh et al., 2006). Additionally, given the variations in the GATA motif reported by Budovskaya et al. (2008), it is possible that more than one GATA factor is modulating expression of these genes; indeed, the GATA factors *egr-1* and *egl-27* were also found to suppress the longevity of *daf-2* mutants (Budovskaya et al., 2008).

PERSPECTIVES: CHALLENGES AND SOLUTIONS

C. elegans DAF-16/FOXO is a key node in a diverse array of physiological processes, including development, aging, immunity, and stress response. Because DAF-16/FOXO plays many roles, its fine-tuned regulation is critical for eliciting appropriate responses in response to different stimuli. As we have described here, recent work has uncovered several conserved interactions that modulate DAF-16 activity in a specific subset of its many functions. While the interactions that regulate DAF-16/FOXO are beginning to be uncovered, more work needs to be done to understand how multiple signals are integrated to elicit an appropriate context-dependent response, and the tissues that are involved in each of these responses must be identified. DAF-16's interactions with co-factors may also specify its choice of transcriptional targets; thus, the identification of those targets, the associated DNA motifs, and the associated co-factors will help uncover the mechanisms underlying the regulation of DAF-16 activity. In addition to standard genetic, biochemical, and expres-

sion microarray techniques, the recent development of such approaches as massively parallel sequencing combined with chromatin immunoprecipitation and high-throughput protein mass spectrometry should help fill these gaps in our knowledge of DAF-16/FOXO interactions.

ACKNOWLEDGMENTS

J.L. is supported by a National Science Foundation Pre-doctoral Fellowship. C.T.M. is a Richard B. Fisher Preceptor in Integrative Genomics, Lewis-Sigler Institute, Princeton, NJ.

REFERENCES

- An JH, Blackwell TK. 2003. SKN-1 links *C. elegans* mesendodermal specification to a conserved oxidative stress response. *Genes Dev* 17:1882–1893.
- Antebi A, Yeh WH, Tait D, Hedgecock EM, Riddle DL. 2000. *daf-12* encodes a nuclear receptor that regulates the dauer diapause and developmental age in *C. elegans*. *Genes Dev* 14:1512–1527.
- Apfeld J, Kenyon C. 1998. Cell nonautonomy of *C. elegans daf-2* function in the regulation of diapause and life span. *Cell* 95:199–210.
- Baugh LR, Sternberg PW. 2006. DAF-16/FOXO regulates transcription of *cki-1*/Cip/Kip and repression of *lin-4* during *C. elegans* L1 arrest. *Curr Biol* 16:780–785.
- Berdichevsky A, Viswanathan M, Horvitz HR, Guarente L. 2006. *C. elegans* SIR-2.1 interacts with 14–3–3 proteins to activate DAF-16 and extend life span. *Cell* 125:1165–1177.
- Berman JR, Kenyon C. 2006. Germ-cell loss extends *C. elegans* life span through regulation of DAF-16 by *kri-1* and lipophilic-hormone signaling. *Cell* 124:1055–1068.
- Blüher M, Kahn BB, Kahn CR. 2003. Extended longevity in mice lacking the insulin receptor in adipose tissue. *Science* 299:572–574.
- Brunet A, Bonni A, Zigmond MJ, Lin MZ, Juo P, Hu LS, Anderson MJ, Arden KC, Blenis J, Greenberg ME. 1999. Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. *Cell* 96:857–868.
- Brunet A, Sweeney LB, Sturgill JF, Chua KF, Greer PL, Lin Y, Tran H, Ross SE, Mostoslavsky R, Cohen HY, Hu LS, Cheng H, Jedrychowski MP, Gygi SP, Sinclair DA, Alt FW, Greenberg ME. 2004. Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase. *Science* 303:2011–2015.
- Budovskaya YV, Wu K, Southworth LK, Jiang M, Tedesco P, Johnson TE, Kim SK. 2008. An *elt-3/elt-5/elt-6* GATA transcription circuit guides aging in *C. elegans*. *Cell* 134:291–303.
- Essers MA, Weijzen S, de Vries-Smits AM, Saarloos I, de Ruiter ND, Bos JL, Burgering BMT. 2004. FOXO transcription factor activation by oxidative stress mediated by the small GTPase Ral and JNK. *EMBO J* 23:4802–4812.
- Essers MAG, de Vries-Smits LMM, Barker N, Polderman PE, Burgering BMT, Korswagen HC. 2005. Functional interaction between β -catenin and FOXO in oxidative stress signaling. *Science* 308:1181–1184.
- Flachsbart F, Caliebe A, Kleindorp R, Blanché H, von Eller-Eberstein H, Nikolaus S, Schreiber S, Nebel A. 2009. Association of FOXO3A variation with human longevity confirmed in German centenarians. *Proc Natl Acad Sci USA* 106:2700–2705.
- Flatt T, Min KJ, D'Alterio C, Villa-Cuesta E, Cumbers J, Lehmann R, Jones DL, Tatar M. 2008. *Drosophila* germ-line modulation of insulin signaling and lifespan. *Proc Natl Acad Sci USA* 105:6368–6373.
- Friedman DB, Johnson TE. 1988. A mutation in the *age-1* gene in *Caenorhabditis elegans* lengthens life and reduces hermaphrodite fertility. *Genetics* 118:75–86.
- Furuyama T, Nakazawa T, Nakano I, Mori N. 2000. Identification of the differential distribution patterns of mRNAs and consensus binding sequences for mouse DAF-16 homologues. *Biochem J* 349:629–634.
- Garigan D, Hsu A, Fraser AG, Kamath RS, Ahringer J, Kenyon C. 2002. Genetic analysis of tissue aging in *Caenorhabditis elegans*: a role for heat-shock factor and bacterial proliferation. *Genetics* 161:1101–1112.
- Garsin DA, Villanueva JM, Begun J, Kim DH, Sifri CD, Calderwood SB, Ruvkun G, Ausubel FM. 2003. Long-lived *C. elegans daf-2* mutants are resistant to bacterial pathogens. *Science* 300:1921.
- Giannakou ME, Goss M, Jnger MA, Hafen E, Leevers SJ, Partridge L. 2004. Long-lived *Drosophila* with overexpressed dFOXO in adult fat body. *Science* 305:361.
- Gottlieb S, Ruvkun, G. 1994. *daf-2*, *daf-16*, and *daf-23*: genetically interacting genes controlling Dauer formation in *Caenorhabditis elegans*. *Genetics* 137:107–120.
- Henderson ST, Johnson TE. 2001. *daf-16* integrates developmental and environmental inputs to mediate aging in the nematode *Caenorhabditis elegans*. *Curr Biol* 11:1975–1980.
- Hertweck M, Gbel C, Baumeister R. 2004. *C. elegans* SGK-1 is the critical component in the Akt/PKB kinase complex to control stress response and life span. *Dev Cell* 6:577–588.
- Honda Y, Honda S. 1999. The *daf-2* gene network for longevity regulates oxidative stress resistance and Mn-superoxide dismutase gene expression in *Caenorhabditis elegans*. *FASEB J* 13:1385–1393.

- Hoogeboom D, Essers MAG, Polderman PE, Voets E, Smits LMM, Burgering BMT. 2008. Interaction of FOXO with β -catenin inhibits β -catenin/T cell factor activity. *J Biol Chem* 283:9224–9230.
- Hsin H, Kenyon C. 1999. Signals from the reproductive system regulate the life-span of *C. elegans*. *Nature* 399:362–366.
- Hsu A, Murphy CT, Kenyon C. 2003. Regulation of aging and age-related disease by DAF-16 and heat-shock factor. *Science* 300:1142–1145.
- Iser WB, Gami MS, Wolkow CA. 2007. Insulin signaling in *Caenorhabditis elegans* regulates both endocrine-like and cell-autonomous outputs. *Dev Biol* 303:434–447.
- Jia K, Thomas C, Akbar M, Sun Q, Adams-Huet B, Gilpin C, Levine B. 2009. Autophagy genes protect against *Salmonella typhimurium* infection and mediate insulin signaling-regulated pathogen resistance. *Proc Natl Acad Sci USA* 106:14564–14569.
- Kaerberlein M, McVey M, Guarente L. 1999. The SIR2/3/4 complex and SIR2 alone promote longevity in *Saccharomyces cerevisiae* by two different mechanisms. *Genes Dev* 13:2570–2580.
- Kawano T, Ito Y, Ishiguro M, Takuwa K, Nakajima T, Kimura Y. 2000. Molecular cloning and characterization of a new insulin/IGF-like peptide of the nematode *Caenorhabditis elegans*. *Biochem Biophys Res Commun* 273:431–436.
- Kenyon C, Murphy CT. 2006. Enrichment of regulatory motifs upstream of predicted DAF-16 targets. *Nat Genet* 38:397–398.
- Kenyon C, Chang J, Gensch E, Rudner A, Tabtiang R. 1993. A *C. elegans* mutant that lives twice as long as wild type. *Nature* 366:461–464.
- Kimura KH, Tissenbaum A, Liu Y, Ruvkun G. 1997. *daf-2*, an insulin receptor-like gene that regulates longevity and diapause in *Caenorhabditis elegans*. *Science* 277:942–946.
- Lee SS, Kennedy S, Tolonen AC, Ruvkun G. 2003. DAF-16 target genes that control *C. elegans* life-span and metabolism. *Science* 300:644–647.
- Lehtinen MK, Yuan Z, Boag PR, Yang Y, Villn J, Becker EB, DiBacco S, de la Iglesia N, Gygi S, Blackwell TK, Bonni A. 2006. A conserved MST-FOXO signaling pathway mediates oxidative-stress responses and extends life span. *Cell* 125:987–1001.
- Li J, Tewari M, Vidal M, Lee SS. 2007. The 14–3–3 protein FTT-2 regulates DAF-16 in *Caenorhabditis elegans*. *Dev Biol* 301:82–91.
- Li J, Ebata A, Dong Y, Rizki G, Iwata T, Lee SS. 2008. *Caenorhabditis elegans* HCF-1 functions in longevity maintenance as a DAF-16 regulator. *PLoS Biol* 6:e233.
- Li W, Kennedy SG, Ruvkun G. 2003. *daf-28* encodes a *C. elegans* insulin superfamily member that is regulated by environmental cues and acts in the DAF-2 signaling pathway. *Genes Dev* 17:844–858.
- Libina N, Berman JR, Kenyon C. 2003. Tissue-specific activities of *C. elegans* DAF-16 in the regulation of lifespan. *Cell* 115:489–502.
- Lin K, Dorman JB, Rodan A, Kenyon C. 1997. *daf-16*: An HNF-3/forkhead family member that can function to double the life-span of *Caenorhabditis elegans*. *Science* 278:1319–1322.
- Lin K, Hsin H, Libina N, Kenyon C. 2001. Regulation of the *Caenorhabditis elegans* longevity protein DAF-16 by insulin/IGF-1 and germline signaling. *Nat Genet* 28:139–145.
- McElwee J, Bubb K, Thomas JH. 2003. Transcriptional outputs of the *Caenorhabditis elegans* forkhead protein DAF-16. *Aging Cell* 2:111–121.
- Mendoza MC, Du F, Iranfar N, Tang N, Ma H, Loomis WF, Firtel RA. 2005. Loss of SMEK, a novel, conserved protein, suppresses *mek1* null cell polarity, chemotaxis, and gene expression defects. *Mol Cell Biol* 25:7839–7853.
- Michael MD, Kulkarni RN, Postic C, Previs SF, Shulman GI, Magnuson MA, Kahn CR. 2000. Loss of insulin signaling in hepatocytes leads to severe insulin resistance and progressive hepatic dysfunction. *Mol Cell* 6:87–97.
- Morley JF, Morimoto RI. 2004. Regulation of longevity in *Caenorhabditis elegans* by heat shock factor and molecular chaperones. *Mol Biol Cell* 15:657–664.
- Motola DL, Cummins CL, Rottiers V, Sharma KK, Li T, Li Y, Suino-Powell K, Xu EH, Auchus RJ, Antebi A, Mangelsdorf DJ. 2006. Identification of ligands for DAF-12 that govern dauer formation and reproduction in *C. elegans*. *Cell* 124:1209–1223.
- Murakami S, Johnson TE. 1996. A genetic pathway conferring life extension and resistance to UV stress in *Caenorhabditis elegans*. *Genetics* 143:1207–1218.
- Murphy CT, McCarrroll SA, Bargmann CI, Fraser A, Kamath RS, Ahringer J, Li H, Kenyon C. 2003. Genes that act downstream of DAF-16 to influence the lifespan of *Caenorhabditis elegans*. *Nature* 424:277–283.
- Murphy CT, Lee S, Kenyon C. 2007. Tissue entrainment by feedback regulation of insulin gene expression in the endoderm of *Caenorhabditis elegans*. *Proc Natl Acad Sci USA* 104:19046–19050.
- Ogg S, Ruvkun G. 1998. The *C. elegans* PTEN homolog, DAF-18, acts in the insulin receptor-like metabolic signaling pathway. *Mol Cell* 2:887–893.
- Ogg S, Paradis S, Gottlieb S, Patterson GI, Lee L, Tissenbaum HA, Ruvkun G. 1997. The Fork head transcription factor DAF-16 transduces insulin-like metabolic and longevity signals in *C. elegans*. *Nature* 389:994–999.
- Oh SW, Mukhopadhyay A, Svrzikapa N, Jiang F, Davis RJ, Tissenbaum HA. 2005. JNK regulates lifespan in *Caenorhabditis elegans* by modulating nuclear translocation of forkhead transcription factor/DAF-16. *Proc Natl Acad Sci USA* 102:4494–4499.
- Oh SW, Mukhopadhyay A, Dixit BL, Raha T, Green MR, Tissenbaum HA. 2006. Identification of direct DAF-16 targets controlling longevity, metabolism and diapause by chromatin immunoprecipitation. *Nature Genetics* 38:251–257.
- Oliveira RP, Abate JP, Dilks K, Landis J, Ashraf J, Murphy CT, Blackwell TK. 2009. Condition-adapted stress and longevity gene regulation by *Caenorhabditis elegans* SKN-1/Nrf. *Aging Cell* 8:524–541.
- Padmanabhan S, Mukhopadhyay A, Narasimhan SD, Tesz G, Czech MP, Tissenbaum HA. 2009. A PP2A regulatory subunit regulates *C. elegans* insulin/IGF-1 signaling by modulating AKT-1 phosphorylation. *Cell* 136:939–951.
- Paradis S, Ruvkun G. 1998. *Caenorhabditis elegans* Akt/PKB transduces insulin receptor-like signals from AGE-1 PI3 kinase to the DAF-16 transcription factor. *Genes Dev* 12:2488–2498.
- Paradis S, Ailion M, Toker A, Thomas JH, Ruvkun G. 1999. A PDK1 homolog is necessary and sufficient to transduce AGE-1 PI3 kinase signals that regulate diapause in *Caenorhabditis elegans*. *Genes Dev* 13:1438–1452.
- Pierce SB, Costa M, Wisotzkey R, Devadhar S, Homburger SA, Buchman AR, Ferguson KC, Heller J, Platt DM, Pasquinelli AA, Liu LX, Doberstein SK, Ruvkun G. 2001. Regulation of DAF-2 receptor signaling by human insulin and *ins-1*, a member of the unusually large and diverse *C. elegans* insulin gene family. *Genes Dev* 15:672–686.
- Rine J, Herskowitz I. 1987. Four genes responsible for a position effect on expression from HML and HMR in *Saccharomyces cerevisiae*. *Genetics* 116:9–22.
- Rogina B, Helfand SL. 2004. Sir2 mediates longevity in the fly through a pathway related to calorie restriction. *Proc Natl Acad Sci USA* 101:15998–16003.
- Singh V, Aballay A. 2006. Heat-shock transcription factor (HSF)-1 pathway required for *Caenorhabditis elegans* immunity. *Proc Natl Acad Sci USA* 103:13092–13097.
- Singh V, Aballay A. 2009. Regulation of DAF-16-mediated innate immunity in *C. elegans*. *J Biol Chem* 103:13092–13097.
- Suh Y, Atzmon G, Cho MO, Hwang D, Liu B, Leahy DJ, Barzilay N, Cohen P. 2008. Functionally significant insulin-like growth factor I receptor mutations in centenarians. *Proc Natl Acad Sci USA* 105:3438–3442.
- Tatar M, Bartke A, Antebi A. 2003. The endocrine regulation of aging by insulin-like signals. *Science* 299:1346–1351.
- Tissenbaum HA, Guarente L. 2001. Increased dosage of a *sir-2* gene extends lifespan in *Caenorhabditis elegans*. *Nature* 410:227–230.
- Tullet JMA, Hertweck M, An JH, Baker J, Hwang JY, Liu S, Oliveira RP, Baumeister R, Blackwell TK. 2008. Direct inhibition of the longevity-promoting factor SKN-1 by insulin-like signaling in *C. elegans*. *Cell* 132:1025–1038.

- van der Horst A, Tertoolen LGJ, de Vries-Smits LMM, Frye RA, Medema RH, Burgering BMT. 2004. FOXO4 is acetylated upon peroxide stress and deacetylated by the longevity protein hSir2(SIRT1). *J Biol Chem* 279:28873–28879.
- van Hemert MJ, Steensma HY, van Heusden GP. 2001. 14–3-3 proteins: key regulators of cell division, signalling and apoptosis. *BioEssays* 23:936–946.
- Vowels JJ, Thomas JH. 1992. Genetic analysis of chemosensory control of dauer formation in *Caenorhabditis elegans*. *Genetics* 130:105–123.
- Wang W, Shakes DC. 1997. Expression patterns and transcript processing of *ftt-1* and *ftt-2*, two *C. elegans* 14–3-3 homologues. *J Mol Biol* 268:619–630.
- Wang Y, Oh SW, Deplancke B, Luo J, Walhout AJ, Tissenbaum HA. 2006. *C. elegans* 14–3-3 proteins regulate life span and interact with SIR-2.1 and DAF-16/FOXO. *Mech Ageing Dev* 127:741–747.
- Wilcox BJ, Donlon TA, He Q, Chen R, Grove JS, Yano K, Masaki KH, Willcox DC, Rodriguez B, Curb JD. 2008. FOXO3A genotype is strongly associated with human longevity. *Proc Natl Acad Sci USA* 105:13987–13992.
- Wolff S, Ma H, Burch D, Maciel GA, Hunter T, Dillin A. 2006. SMK-1, an essential regulator of DAF-16-mediated longevity. *Cell* 124:1039–1053.
- Wolkow CA, Kimura KD, Lee MS, Ruvkun G. 2000. Regulation of *C. elegans* life-span by insulinlike signaling in the nervous system. *Science* 290:147–150.
- Wood JG, Rogina B, Lavu S, Howitz K, Helfand SL, Tatar M, Sinclair D. 2004. Sirtuin activators mimic caloric restriction and delay ageing in metazoans. *Nature* 430:686–689.
- Yan L, Lavin VA, Moser LR, Cul Q, Kanles C, Yang E. 2008. PP2A regulates the proapoptotic activity of FOXO1. *J Biol Chem* 283:7411–7420.