Cell

A Genetic Program Promotes *C. elegans* Longevity at Cold Temperatures via a Thermosensitive TRP Channel

Rui Xiao,[1] Bi Zhang,[1,3,5] Yongming Dong,[1,5] Jianke Gong,[1,3,5] Tao Xu,[3,4] Jianfeng Liu,[3] and X.Z. Shawn Xu[1,2,*]
[1]Life Sciences Institute
[2]Department of Molecular and Integrative Physiology
University of Michigan, Ann Arbor, MI 48109, USA
[3]College of Life Science and Technology, Huazhong University of Science and Technology, Wuhan, Hubei 430074, China
[4]Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China
[5]These authors contributed equally to this work
*Correspondence: shawnxu@umich.edu
http://dx.doi.org/10.1016/j.cell.2013.01.020

SUMMARY

Both poikilotherms and homeotherms live longer at lower body temperatures, highlighting a general role of temperature reduction in lifespan extension. However, the underlying mechanisms remain unclear. One prominent model is that cold temperatures reduce the rate of chemical reactions, thereby slowing the rate of aging. This view suggests that cold-dependent lifespan extension is simply a passive thermodynamic process. Here, we challenge this view in *C. elegans* by showing that genetic programs actively promote longevity at cold temperatures. We find that TRPA-1, a cold-sensitive TRP channel, detects temperature drop in the environment to extend lifespan. This effect requires cold-induced, TRPA-1-mediated calcium influx and a calcium-sensitive PKC that signals to the transcription factor DAF-16/FOXO. Human TRPA1 can functionally substitute for worm TRPA-1 in promoting longevity. Our results reveal a previously unrecognized function for TRP channels, link calcium signaling to longevity, and, importantly, demonstrate that genetic programs contribute to lifespan extension at cold temperatures.

INTRODUCTION

Aging can be modulated by both environmental and genetic factors (Fontana et al., 2010; Kenyon, 2010). Work in model organisms, such as yeast, worms, flies, and mice, has identified a number of avenues that promote longevity. For example, restricting food intake (dietary restriction or DR), decreasing insulin/IGF-1 signaling (IIS), slowing mitochondrial respiration, reducing germline function, or lowering temperature can all extend lifespan (Kenyon, 2010). Over the past two decades, studies in model organisms have led to an increasingly clear understanding of how DR, IIS, mitochondrial function, and the reproductive system modulate longevity (Fontana et al., 2010; Kenyon, 2010). By contrast, very little is known about how temperature regulates lifespan (Conti, 2008).

The phenomenon that poikilotherms (e.g., worms, flies, and fish) have a longer lifespan at lower temperatures was first documented nearly a century ago (Loeb and Northrop, 1916). Recent work demonstrates that lowering the core body temperature of homeothermic animals, such as mice, also increases lifespan (Conti et al., 2006), highlighting a general role of temperature reduction in lifespan extension in both poikilotherms and homeotherms. In *C. elegans*, raising the culturing temperature (e.g., to 25°C) greatly shortens lifespan, which is counteracted by the AFD neuron via neuroendocrine signals (Lee and Kenyon, 2009). Conversely, rearing worms at low temperatures (e.g., 20°C and 15°C) greatly lengthens lifespan (Klass, 1977; Wu et al., 2009). However, the mechanisms underlying this cold-dependent lifespan extension remain unclear. One prominent model is that lowering the body temperature would reduce the rate of chemical reactions, thereby leading to a slower pace of living. This model suggests that the extended lifespan observed at low temperatures is simply a passive thermodynamic process. Indeed, it takes a longer time for worms to develop from embryos to adults at lower temperatures, a phenomenon seemingly consistent with this model.

Here, we sought to challenge this view in *C. elegans*. We reasoned that if genetic programs can actively promote longevity under other paradigms, they might also do so at cold temperatures. We found that TRPA-1, a cold-sensitive TRP channel, can act as a thermosensor to detect temperature drop in the environment to initiate a genetic program to extend lifespan. This program includes TRPA-1, TRPA-1-mediated calcium influx, PKC-2 (a calcium-sensitive PKC), SGK-1 (a DAF-16/FOXO kinase), and the FOXO transcription factor DAF-16, which is a key regulator of lifespan. We also show that the intestine, a nonexcitable tissue known to be a signaling hub for lifespan regulation, is in fact cold sensitive and responds to temperature decreases in the environment. This finding expands the repertoire of cold receptors from neurons to nonexcitable cells. Interestingly, human TRPA1 can functionally substitute for worm TRPA-1 in lifespan extension in a temperature- and

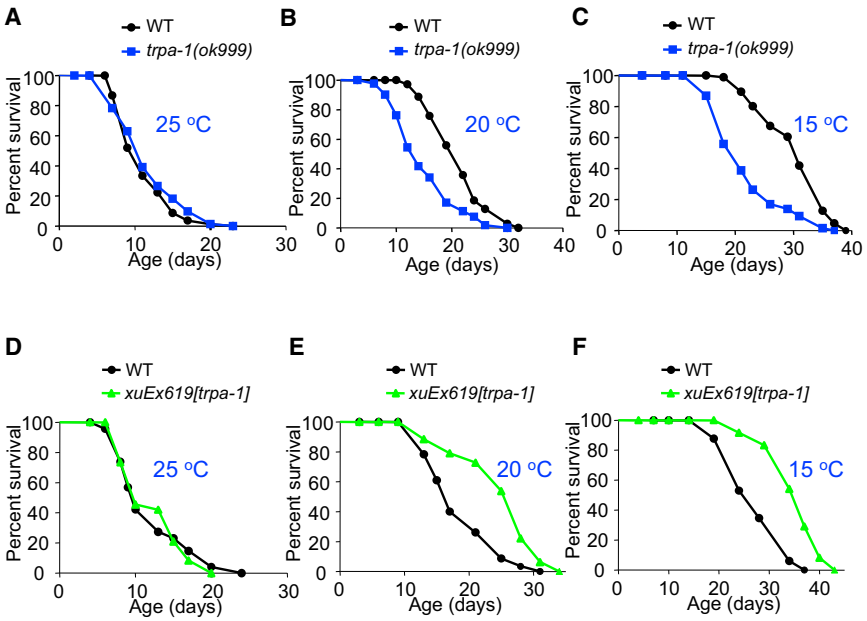


Figure 1. TRPA-1 Promotes Longevity at Cold but Not Warm Temperatures

(A–C) *trpa-1* null mutant worms are short lived at 20°C (B) and 15°C (C) but not at 25°C (A).

(D and E) Overexpression of wild-type (WT) *trpa-1* gene extends lifespan at 20°C and 15°C but not at 25°C. Transgenic worms express full-length *trpa-1* genomic DNA.

See also Figure S1 and Table S1.

agonist-dependent manner. Our studies demonstrate that genetic programs actively contribute to lifespan extension at cold temperatures, identify an unexpected function for TRP family channels and intestinal cells, and also reveal a functional link between calcium signaling and longevity.

RESULTS

Loss of TRPA-1 Shortens Lifespan at Cold but Not Warm Temperatures

We hypothesized that genetic programs may actively promote longevity at cold temperatures. As a first step to test this idea, we envisioned that a cold sensor(s) may detect temperature decreases in the environment to initiate a pro-longevity genetic program. The best known cold sensors in mammals are the TRP-family channels TRPA1 and TRPM8, but TRPM8 does not have a homolog in *C. elegans* (McKemy et al., 2002; Peier et al., 2002; Story et al., 2003; Venkatachalam and Montell, 2007). TRPA-1, the *C. elegans* ortholog of the mammalian TRPA1 channel, thus came to our attention (Kindt et al., 2007; Venkatachalam and Montell, 2007; Xiao and Xu, 2009).

As is the case with its mammalian counterparts (Karashima et al., 2009; Story et al., 2003), TRPA-1 is also a cold-sensitive channel, opening when temperature drops to ~20°C (Chatzigeorgiou et al., 2010). Three temperatures (i.e., 15°C, 20°C, and 25°C) are common laboratory conditions for culturing *C. elegans*. If TRPA-1 is involved in promoting longevity at low temperatures, one would expect that mutant worms lacking TRPA-1 should have a shorter lifespan at 20°C and 15°C but not at 25°C than do wild-type worms. This is because this cold-sensitive channel is expected to be functional at 20°C and 15°C but remains closed at 25°C. Consistent with this prediction, we found that *trpa-1* null mutant worms showed a significantly shorter lifespan than did wild-type worms at 20°C (Figure 1B; Table S1 available online). A similar phenomenon was also observed at 15°C (Figure 1C). By contrast, at 25°C, the lifespan of *trpa-1* mutant worms was similar to that of wild-type (Figure 1A). No notable defect in development or fecundity was detected in *trpa-1* mutant worms (Figures S1A and S1B). These results suggest that TRPA-1 may function to extend lifespan at cold but not warm temperatures. This cold-dependent pro-longevity effect is opposite to that of other sensory channels, such as TAX-2, TAX-4, and OCR-2, all of which appear to shorten lifespan at low temperatures (20°C) (Apfeld and Kenyon, 1999; Lee and Ashrafi, 2008; Lee and Kenyon, 2009).

Transgenic Expression of TRPA-1 Extends Lifespan at Cold but Not Warm Temperatures

A short-lived mutant phenotype, however, does not provide sufficient evidence that the gene of interest promotes longevity. We therefore performed the converse experiment by overexpressing wild-type TRPA-1 in worms. Transgenic expression of TRPA-1 under its own promoter increased lifespan at 20°C and 15°C but not at 25°C (Figures 1D–1F). This provides further evidence that TRPA-1 can promote longevity at low temperatures. As TRPA-1 is functional at 20°C, for simplicity, we focused on this temperature for further characterizations.

We next examined in which tissues TRPA-1 acts to promote longevity. TRPA-1 is known to be expressed in multiple tissues, including neurons, muscles, hypodermal cells, and the intestine (Dupuy et al., 2007; Kindt et al., 2007) (Figure S1C). Expression of TRPA-1 in intestinal cells or neurons was sufficient to extend the lifespan of wild-type worms, with intestinal expression showing the most robust effect (Figure 2A). By contrast, expression of TRPA-1 in muscles or hypodermal cells did not extend lifespan (Figure 2A). This suggests that TRPA-1 can function in both intestinal cells and neurons to modulate lifespan. Notably, the intestine and nervous system are the two tissues where the transcription factor DAF-16/FOXO, a key regulator of lifespan, acts to promote longevity (Libina et al., 2003; Lin et al., 1997; Ogg et al., 1997). As intestinal expression of TRPA-1 displayed the strongest effect in lifespan extension, we focused on this tissue for further characterizations.

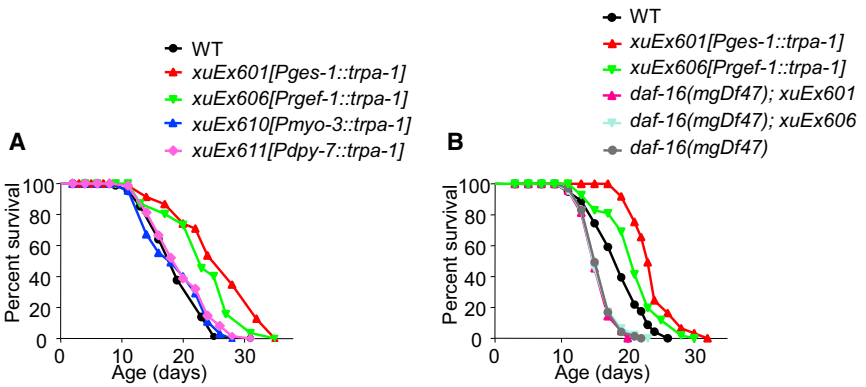


Figure 2. TRPA-1-Dependent Lifespan Extension Requires DAF-16

(A) Transgenic expression of *trpa-1* in the intestine and neurons, but not in muscles or hypodermal cells, extends lifespan. The *ges-1, rgef-1, myo-3,* and *dpy-7* promoters were used to drive expression of *trpa-1* complementary DNA (cDNA) in the intestine, neurons, muscles, and hypodermal cells, respectively (Aamodt et al., 1991; Altun-Gultekin et al., 2001; Fire and Waterston, 1989; Gilleard et al., 1997).

(B) Loss of *daf-16* fully suppresses the long-lived phenotype of *trpa-1* transgenic worms.

Lifespan: 20°C. See also Figure S2 and Table S1.

TRPA-1 Acts upstream of the FOXO Transcription Factor DAF-16

Clearly, as a cold sensor, TRPA-1 cannot promote longevity on its own. We thus sought to identify transcription factors that act downstream of TRPA-1 to promote longevity, as transcription factors are known to be the master regulators of lifespan in *C. elegans* (Kenyon, 2010). Loss of *daf-16* abolished the ability of *trpa-1* transgenes to extend lifespan (Figure 2B). In addition, *daf-16* RNA interference (RNAi) abrogated the temperature sensitivity of *trpa-1* mutant worms (Figures S2A and S2B). Furthermore, overexpression of DAF-16 rescued the short-lived phenotype of *trpa-1* mutant worms at low temperatures (Figure S2C). These findings together strongly suggest that TRPA-1 acts upstream of DAF-16. By contrast, *trpa-1* transgene can still extend the lifespan of worms deficient in several other known pro-longevity transcription factors, such as SKN-1/Nrf, HSF-1, and PHA-4/FOXA (Figures S2D and S2F) (Bishop and Guarente, 2007; Hsu et al., 2003; Panowski et al., 2007; Tullet et al., 2008). Similarly, the pro-longevity effect of *trpa-1* transgene was also independent of the transcription factor DAF-12 (a nuclear hormone receptor) and its regulator DAF-9 (Figures S2G and S2H) (Antebi et al., 2000; Jia et al., 2002). Although these results do not exclude the involvement of other transcription factors, they demonstrate that DAF-16 is required for the function of TRPA-1 in lifespan extension, suggesting that DAF-16 acts downstream of TRPA-1.

TRPA-1-Mediated Lifespan Extension Requires Calcium and a Calcium-Sensitive PKC

As an ion channel, TRPA-1 is unlikely to directly signal to DAF-16. We thus set out to identify additional components in the TRPA-1- and DAF-16-dependent genetic pathway. TRPA proteins are calcium-permeable nonselective cation channels (Jordt et al., 2004; Story et al., 2003), and their ion selectivity has been well characterized (Wang et al., 2008). A highly conserved D/E residue in the putative selectivity filter of TRPA channels is essential for calcium permeability (Wang et al., 2008). Mutating this residue to A does not affect the overall function of the channel but selectively renders the channel impermeable to Ca^{2+} without affecting its permeability to Na^{+} or K^{+} (Wang et al., 2008). We found that TRPA-1 harboring this point mutation (i.e., E1018A) failed to extend lifespan in transgenic worms (Figure 3A). This hints at a critical role for calcium in TRPA-1-mediated lifespan extension.

Then how might calcium transmit signals to DAF-16? The function of DAF-16 is best known to be regulated by kinases. We thus asked whether any calcium-sensitive kinase can transmit calcium signals from TRPA-1 to DAF-16.

To meet the first requirement, the candidate gene should act downstream of TRPA-1. The best known calcium-sensitive kinases are probably CaMKs. *C. elegans* encodes two CaMKs: CMK-1 and UNC-43 (Reiner et al., 1999; Satterlee et al., 2004). We therefore checked *cmk-1* and *unc-43* mutant worms but found that null mutations of these two CaMKs failed to suppress the long-lived phenotype conferred by *trpa-1* transgene (Figures S3A and S3B), indicating that both CaMKs are not required for the function of TRPA-1 in promoting longevity. Another major class of calcium-sensitive kinases is PKC. The *C. elegans* genome encodes four PKCs; however, PKC-2 represents the sole classical PKC (cPKC) and also the only PKC equipped with a C2 domain that is known to confer calcium sensitivity to this type of kinase (Islas-Trejo et al., 1997). Importantly, PKC-2 has been demonstrated to be directly activated by calcium in vitro (Islas-Trejo et al., 1997). We found that loss of PKC-2 fully suppressed the long-lived phenotype of *trpa-1* transgenic animals, indicating that PKC-2 is required for the function of TRPA-1 in the pathway (Figure 3B). These data suggest that PKC-2 may act downstream of TRPA-1 and calcium to promote longevity.

PKC-2 Acts downstream of TRPA-1 but upstream of DAF-16 to Extend Lifespan

If PKC-2 indeed resides downstream of TRPA-1 and calcium to promote longevity, animals lacking PKC-2 should be short lived. We thus scored the lifespan of *pkc-2* mutant worms at different temperatures. Remarkably, worms lacking PKC-2 lived significantly shorter than wild-type worms at low (20°C and 15°C) but not warm (25°C) temperatures (Figures 3C–3E). No notable deficit in development or fecundity was detected in these mutant animals (Figures S1A and S1B). This temperature-dependent lifespan phenotype is very similar to that observed in *trpa-1* mutant worms, providing additional evidence that PKC-2 transmits signals from TRPA-1 and calcium.

We further reasoned that if PKC-2 truly acts in the same pathway to promote longevity, its overexpression ought

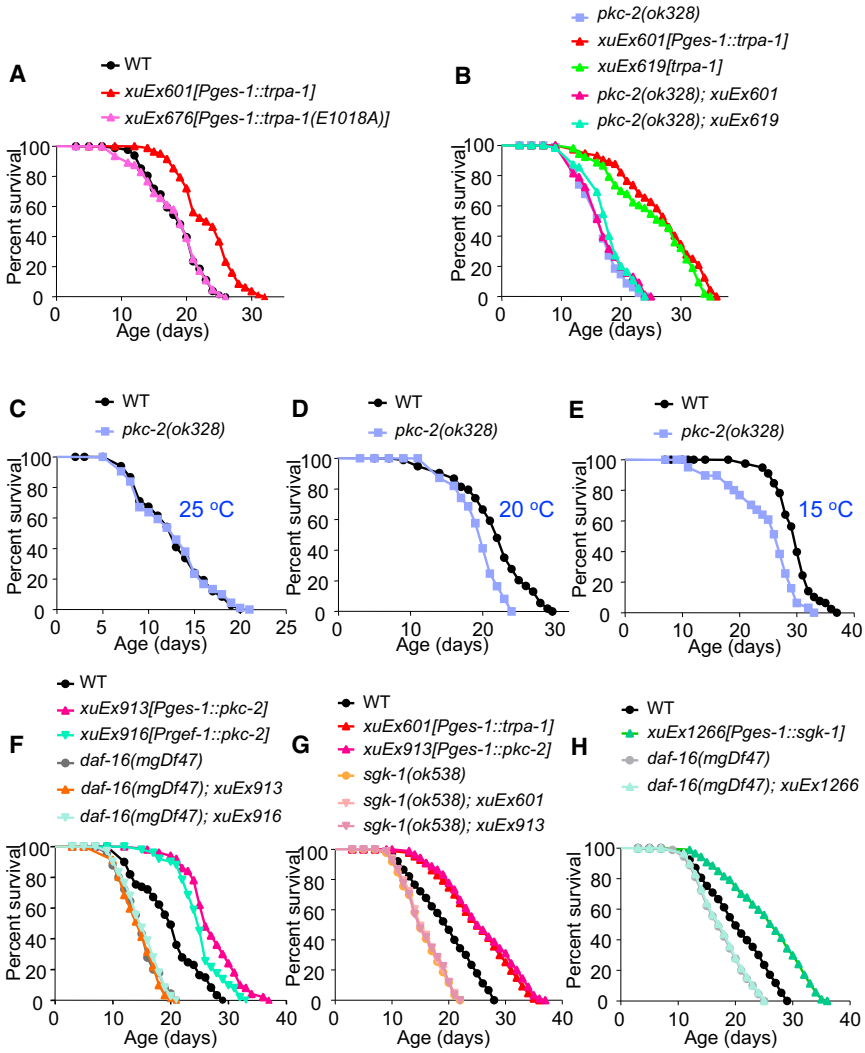


Figure 3. TRPA-1-Dependent Lifespan Extension Requires Calcium and PKC-2

(A) TRPA-1^{E1018A} fails to extend lifespan. Lifespan: 20°C.

(B) Loss of *pkc-2* fully suppresses the long-lived phenotype of *trpa-1* transgenic worms. Lifespan: 20°C.

(C–E) *pkc-2* mutant worms are short lived at 20°C and 15°C but not at 25°C.

(F) Overexpression of *pkc-2* cDNA extends lifespan, which can be fully suppressed by loss of *daf-16*. Lifespan: 20°C.

(G) Loss of *sgk-1* fully suppresses the long-lived phenotype of *trpa-1* and *pkc-2* transgenic animals. Lifespan: 20°C.

(H) Overexpression of *sgk-1* extends lifespan, which can be fully suppressed by loss of *daf-16*. Lifespan: 20°C. *sgk-1* cDNA was expressed as a transgene in the intestine, a tissue in which endogenous *sgk-1* gene is enriched (Hertweck et al., 2004). Lifespan: 20°C.

See also Figure S3 and Table S1.

to extend lifespan. Similar to TRPA-1, PKC-2 is expressed in multiple tissues, including intestinal cells and neurons (Islas-Trejo et al., 1997). Indeed, transgenic expression of PKC-2 in these two tissues can extend lifespan, with intestinal expression showing a more robust effect (Figure 3F). Unlike that with TRPA-1, this effect is temperature independent (not shown). This can be explained by the fact that PKC-2 is not the initial thermosensor, and it is common that overexpression of a downstream effector often bypasses its upstream genes. Indeed, *pkc-2* transgene can fully suppress the short-lived phenotype of *trpa-1* mutant worms, consistent with the view that PKC-2 acts downstream of TRPA-1 (Figure S3C). Moreover, the short-lived phenotype of *trpa-1; pkc-2* double mutant at low temperatures was similar to those of *trpa-1* and *pkc-2* single mutants (Figures S3D–S3F). Thus, the effect of TRPA-1 and PKC-2 on lifespan is not additive, providing further evidence that they act in the same pathway. These observations together strongly suggest that PKC-2 acts downstream of TRPA-1 and calcium to promote longevity at low temperatures.

We then asked whether PKC-2 acts upstream of DAF-16 to extend lifespan. We found that loss of *daf-16* abolished the long-lived phenotype of *pkc-2* transgenic worms, indicating that DAF-16 is required for the function of PKC-2 in promoting longevity (Figure 3F). As was the case with *trpa-1* mutant, *daf-16* RNAi also abrogated the temperature sensitivity of *pkc-2* mutant worms (Figures S2A and S2B). Furthermore, overexpression of DAF-16 rescued the short-lived phenotype of *pkc-2* mutant worms at low temperatures (Figure S2C). These observations together strongly suggest that PKC-2 functions upstream of DAF-16 to extend lifespan.

TRPA-1- and PKC-2-Dependent Lifespan Extension Requires SGK-1, a DAF-16 Kinase

How does PKC-2 signal to DAF-16? Given that DAF-16 is known to be regulated by phosphorylation, the simplest model would be that PKC-2 may signal to DAF-16 by directly phosphorylating DAF-16. However, we failed to detect phosphorylation of DAF-16 by PKC-2 in in vitro kinase assays (not shown). Though this does not rule out the possibility that PKC-2 may phosphorylate DAF-16 in vivo, it strongly suggests that PKC-2 probably regulates DAF-16 indirectly.

Because DAF-16 is best known to be regulated by kinases, and PKC-2 is probably not a DAF-16 kinase, we reasoned that another kinase may be present in the TRPA-1 pathway to regulate DAF-16, probably by acting downstream of PKC-2. We therefore considered those known DAF-16 kinases, including AKT-1, AKT-2, JNK-1, and SGK-1 (Hertweck et al., 2004; Lin et al., 2001; Oh et al., 2005; Paradis and Ruvkun, 1998).

trpa-1 transgene can further extend the lifespan of *akt-1, akt-2*, and *jnk-1* mutant worms, suggesting that these three DAF-16 kinases are probably not in the TRPA-1 pathway (Figures S3G–S3I). By contrast, loss of SGK-1 completely suppressed TRPA-1- and PKC-2-dependent lifespan extension (Figure 3G), suggesting that SGK-1 is probably a member of the TRPA-1 pathway and acts downstream of TRPA-1 and PKC-2.

SGK-1 Acts downstream of TRPA-1 and PKC-2 but upstream of DAF-16 to Extend Lifespan

A previous study reported that RNAi knockdown of *sgk-1* extends lifespan, suggesting that the function of this gene is to shorten lifespan (Hertweck et al., 2004). More recent studies using a *sgk-1* deletion mutant, however, demonstrated that loss of *sgk-1* shortens lifespan (Alam et al., 2010; Soukas et al., 2009). We also found that *sgk-1* mutant animals were short lived (Figure 3G). The short-lived phenotype of *sgk-1* mutants suggests that the function of SGK-1 is to promote longevity. Indeed, overexpression of SGK-1 extended lifespan (Figure 3H). No temperature dependence was observed in *sgk-1* worms (not shown), consistent with the notion that SGK-1 is not a temperature sensor on its own. SGK-1 has been suggested to act upstream of DAF-16 (Alam et al., 2010; Hertweck et al., 2004). Indeed, loss of DAF-16 completely suppressed the long-lived phenotype of *sgk-1* transgenic worms (Figure 3H). Conversely, overexpression of DAF-16 rescued the short-lived phenotype of *sgk-1* mutant worms (Figure S2C). These data support the view that SGK-1 acts upstream of DAF-16 to positively regulate its activity. As expected, SGK-1 overexpression also fully rescued the short-lived phenotype of *trpa-1* and *pkc-2* mutant animals, providing additional evidence that SGK-1 acts downstream of TRPA-1 and PKC-2 (Figure S3J). These genetic data, together with the fact that SGK-1 directly phosphorylates DAF-16, place SGK-1 downstream of TRPA-1 and PKC-2 but upstream of DAF-16.

Taken together, our data suggest a mode in which TRPA-1, calcium, PKC-2, SGK-1, and DAF-16 form a signaling pathway to promote longevity at low temperatures.

The TRPA-1 Pathway Does Not Promote Nuclear Translocation of DAF-16

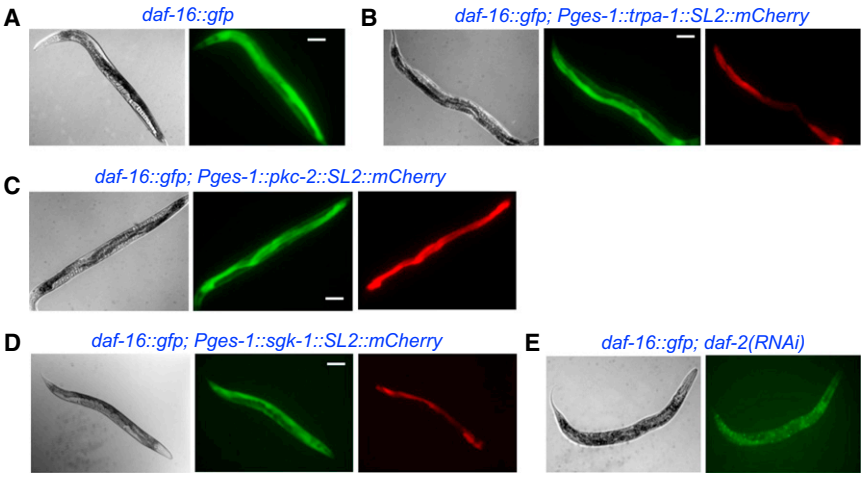
How does the TRPA-1 pathway regulate the function of DAF-16? DAF-16 kinases, such as AKT-1, AKT-2, and JNK-1, are known to regulate nuclear translocation of DAF-16 (Henderson and Johnson, 2001; Lin et al., 2001; Oh et al., 2005; Paradis and Ruvkun, 1998), whereas some other DAF-16 regulators (e.g., HCF-1, EAK-7, and SMK-1) exert their effects by modulating DAF-16 nuclear activity (Alam et al., 2010; Li et al., 2008; Wolff et al., 2006). Using three different quantification methods, we found that the subcellular localization pattern of DAF-16::GFP fusion, which is encoded by the transgene *zIs356* (Henderson and Johnson, 2001), was not affected by *trpa-1*, *pkc-2*, or *sgk-1* transgenes (Figures 4A–4F and S4A–S4G). We also did not detect any notable change in DAF-16 subcellular localization pattern in *sgk-1* mutant worms (Figure 4F). These data demonstrate that unlike other DAF-16 kinases, SGK-1 does not regulate DAF-16 localization. Thus, the TRPA-1 pathway does not appear to promote nuclear translocation of DAF-16.

As a positive control, inactivation of *daf-2*, which encodes the worm insulin/IGF-1 receptor homolog (Kimura et al., 1997), resulted in a marked translocation of DAF-16 to the nucleus (Figures 4E, 4F, S4B, S4F, and S4G). These data are expected, as IIS is well known to regulate nuclear translocation of DAF-16 (Henderson and Johnson, 2001; Lin et al., 2001). This also raises the possibility that the TRPA-1 pathway and IIS may act in parallel to regulate DAF-16. Consistent with this model, transgenic expression of TRPA-1 can further extend the lifespan of *daf-2(e1370)* mutant worms (Figure S4H), as well as worms lacking *akt-1* and *akt-2,* two key components of IIS (Paradis and Ruvkun, 1998) (Figures S3G and S3H). On the other hand, because *daf-2* null alleles are lethal, we are unable to further test this model; thus, the possibility that the two act in an overlapping or the same pathway cannot be formally ruled out. Nevertheless, these data demonstrate that the TRPA-1 pathway does not alter the subcellular localization of DAF-16, suggesting that this pathway may regulate DAF-16 function by promoting its nuclear activity.

The TRPA-1 Pathway Promotes the Nuclear Activity of DAF-16

To gather additional evidence to support a role for the TRPA-1 pathway in promoting the nuclear activity of DAF-16, we performed further experiments. In wild-type worms, DAF-16 protein is distributed in both the cytosol and the nucleus at resting states (Henderson and Johnson, 2001; Lin et al., 2001) (also see Figures S4A and S4F), and it is known that nuclear translocation alone is insufficient to activate DAF-16 (Lin et al., 2001). We reasoned that if the TRPA-1 pathway promotes DAF-16 nuclear activity, then increasing the amount of DAF-16 protein inside the nucleus should potentiate the effect of the TRPA-1 pathway in extending lifespan. To test this, we took advantage of DAF-16^{AM}::GFP, a genetically engineered form of DAF-16 that is functional but that IIS fails to sequester in the cytosol (Lin et al., 2001). Consequently, DAF-16^{AM}::GFP constitutively translocates to the nucleus, leading to an enrichment of DAF-16 within the nucleus (Lin et al., 2001). Because of this nuclear enrichment of DAF-16, one would expect that TRPA-1, PKC-2, and SGK-1 should be more potent in promoting lifespan in this strain. Indeed, *trpa-1, pkc-2*, and *sgk-1* transgenes extended the lifespan of worms containing DAF-16^{AM}::GFP to a greater extent than that of worms expressing DAF-16::GFP (Figures 4G–4I), further suggesting that the TRPA-1 pathway promotes the nuclear activity of DAF-16. Interestingly, in the absence of *trpa-1*, *pkc-2*, and *sgk-1* transgenes, DAF-16^{AM}::GFP itself did not extend lifespan when compared to DAF-16::GFP (Figures 4G–4I), consistent with the view that nuclear translocation alone is insufficient to activate DAF-16 (Lin et al., 2001). These data provide additional evidence that the TRPA-1 pathway promotes DAF-16 nuclear activity.

To provide further evidence, we assayed DAF-16 targets. If the TRPA-1 pathway promotes DAF-16 nuclear activity, one would expect that this pathway should regulate the expression level of DAF-16 target genes. We first examined the expression level of *sod-3*, which is a direct transcriptional target of DAF-16 and



F

Strain	% of total animals assayed				n number
	0% cells nuclear	<50% cells nuclear	>50% cells nuclear	All nuclear	
daf-16::gfp	74.02	25.98	0	0	127
daf-16::gfp; Pges-1::trpa-1	75.00	25.00	0	0	124
daf-16::gfp; Pges-1::pkc-2	79.84	20.16	0	0	124
daf-16::gfp; Pges-1::sgk-1	74.50	25.50	0	0	102
sgk-1(ok538)	75.00	25.00	0	0	100
daf-16::gfp; daf-2(RNAi)	3.97	31.75	20.63	43.65	126

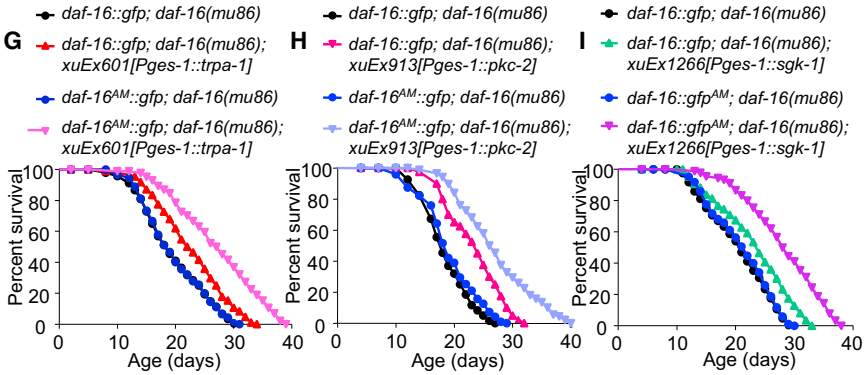


Figure 4. The TRPA-1 Pathway Does Not Alter the Subcellular Localization of DAF-16 but instead Promotes Its Nuclear Activity

(A) DAF-16::GFP is localized to the cytoplasm as well as the nucleus. Sample images: left: DIC; right: DAF-16::GFP. See Figure S4 for images of higher magnification.

(B) *trpa-1* transgene does not alter the localization pattern of DAF-16::GFP. Sample images: left: DIC; middle: DAF-16::GFP; right: mCherry fluorescence marking *trpa-1* transgene. This *trpa-1* transgene (*xuEx785*) also extended lifespan: 23.1 ± 0.5 days versus WT control: 18.9 ± 0.6 days; $p < 0.001$ (log-rank).

(C) *pkc-2* transgene does not alter the localization pattern of DAF-16::GFP. This *pkc-2* transgene (*xuEx911*) also extended lifespan: 26.6 ± 0.5 days versus WT control: 19.8 ± 0.7 days; $p < 0.001$ (log-rank).

(D) *sgk-1* transgene does not alter the localization pattern of DAF-16::GFP. This transgene is the same as that used in Figure 3H.

(E) Nuclear translocation of DAF-16::GFP in *daf-2(RNAi)* worms. The fluorescent puncta in the image label the cell nuclei of the worm (Henderson and Johnson, 2001). See Figure S4B for an image of higher magnification.

(F) Table summarizing the data in panels A–D. We grouped animals into four categories based on the localization pattern of DAF-16::GFP in intestinal cells: 0% cells nuclear, <50% cells nuclear, >50% cells nuclear, and all nuclear, with each representing the percentage of cells showing nuclear enrichment of DAF-16::GFP. There is no significant difference between WT (*daf-16::gfp*) and *trpa-1* or *pkc-2* transgenic worms (ANOVA test).

(G–I) TRPA-1, PKC-2, and SGK-1 promote the lifespan of worms expressing DAF-16^{AM}::GFP to a greater extent than that of worms expressing DAF-16::GFP. Extension of mean lifespan by *trpa-1* transgene in DAF-16^{AM}::GFP and DAF-16::GFP worms is 38% versus 16%, respectively. Extension of mean lifespan by *pkc-2* transgene in DAF-16^{AM}::GFP and DAF-16::GFP worms is 41% versus 26%, respectively. Extension of mean lifespan by *sgk-1* transgene in DAF-16^{AM}::GFP and DAF-16::GFP worms is 34% versus 13%, respectively. Lifespan: 20°C. All scale bars: 100 μm. See also Figure S4 and Table S1.

also a widely used molecular reporter for DAF-16 activity (Lee et al., 2003; Murphy et al., 2003; Oh et al., 2006). We found that SOD-3::GFP fusion protein, which is encoded by the transgene *muIs84* (Libina et al., 2003), was markedly upregulated in *trpa-1*, *pkc-2*, and *sgk-1* transgenic worms (Figures 5A–5E). We then examined the messenger RNA (mRNA) level of endogenous *sod-3* gene as well as five other DAF-16 target genes, including *dod-3, mtl-1, dod-24, dod-22,* and *ins-7* (Lee et al., 2003; Murphy et al., 2003). Among these six target genes, three (*sod-3, mtl-1*, and *dod-3*) are known to be upregulated by DAF-16, whereas the other three (*dod-24, dod-22*, and *ins-7*) are downregulated by DAF-16. We found that overexpression of *trpa-1, pkc-2*, or *sgk-1* significantly potentiated the mRNA level of *sod-3, mtl-1*, and *dod-3*, whereas loss of *trpa-1, pkc-2*, or *sgk-1* led to a significant reduction in the expression of these genes (Figure 5F), indicating that the TRPA-1 pathway regulates the expression level of DAF-16 targets that are upregulated by DAF-16. Likewise, *dod-24, dod-22*, and *ins-7*, which are known to be repressed by DAF-16, were downregulated in *trpa-1, pkc-2*, and *sgk-1* transgenic worms (Figure 5G). Conversely, in *trpa-1, pkc-2*, and *sgk-1* mutant worms, these three DAF-16 target genes became upregulated (Figure 5G). Thus, the TRPA-1 pathway also regulates the expression level of DAF-16 target genes that are repressed by DAF-16. Furthermore, regulation of DAF-16 target genes by *trpa-1, pkc-2*, and *sgk-1* transgenes was DAF-16 dependent (Figure S5). These results together provide further evidence that the TRPA-1 pathway promotes DAF-16 nuclear activity.

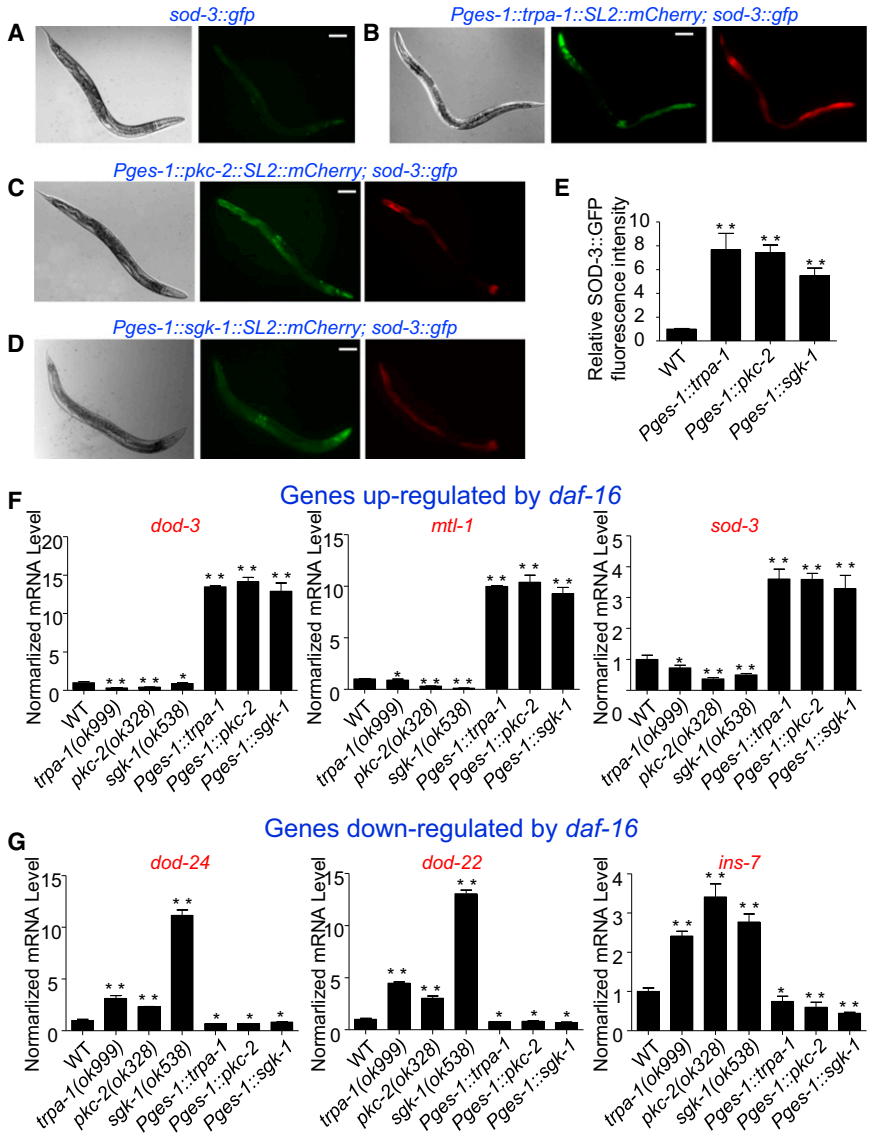


Figure 5. The TRPA-1 Pathway Regulates the Expression Level of DAF-16 Target Genes

(A) SOD-3::GFP is expressed at low levels in WT worms. Left panel: DIC. Right panel: SOD-3::GFP. Scale bars: 100 μm.

(B) *trpa-1* transgene potentiates the expression of SOD-3::GFP. Left: DIC. Middle: SOD-3::GFP. Right: mCherry fluorescence marking *trpa-1* transgene.

(C) *pkc-2* transgene potentiates the expression of SOD-3::GFP. Left: DIC. Middle: SOD-3::GFP. Right: mCherry fluorescence marking *pkc-2* transgene.

(D) *sgk-1* transgene potentiates the expression of SOD-3::GFP. Left: DIC. Middle: SOD-3::GFP. Right: mCherry fluorescence marking *sgk-1* transgene.

(E) Quantification of SOD-3::GFP fluorescence intensity. $n \geq 15$. Error bars: standard error of the mean (SEM). **$p < 0.0001$ (ANOVA with Bonferroni test).

(F and G) qPCR analysis of target genes regulated by DAF-16. qPCR reactions were run in triplicates for each genotype. Each experiment was repeated three times. Worms were reared at 20°C. Error bars: SEM. *$p < 0.05$; **$p < 0.005$ (ANOVA with Bonferroni test).

See also Figure S5.

Cold Evokes a Robust TRPA-1-Dependent Calcium Response in the Intestine, a Signaling Hub for Lifespan Regulation

Having obtained genetic evidence supporting a role for TRPA-1 and calcium signaling in cold-dependent lifespan extension, we then sought to gather physiological evidence for this model. Though it has been well established that certain types of neurons can detect temperature drop in many organisms (Chatzigeorgiou et al., 2010; Gallio et al., 2011; Story et al., 2003), whether the intestine, a nonexcitable tissue, can function as a thermoreceptor has not been explored. Notably, worm intestine is known to be a signaling hub that integrates multiple pro- and antiaging cues to regulate lifespan (Kenyon, 2010). The observation that expression of TRPA-1 in the intestine or neurons was sufficient to extend lifespan prompted us to test whether intestinal cells can function as a cold receptor as do neurons. As it has not been possible to patch the intestine in vivo, we took a calcium-imaging approach to examine the sensitivity of this tissue to cold temperatures. To do so, we expressed G-CaMP, a genetically encoded calcium sensor (Nakai et al., 2001), as a transgene in the intestine. Remarkably, intestinal cells exhibited a robust increase in calcium level in response to cooling (Figures 6A and 6B). This cold-evoked calcium response was also found in the intestine dissected out of the animal, indicating that the response originated from the intestine and thus shall be an intrinsic feature of this tissue (Figure S6). Importantly, such a calcium response was greatly reduced in *trpa-1* mutant worms, consistent with an important role for TRPA-1 in cold-dependent lifespan extension (Figures 6A and 6B). In addition, expression of wild-type TRPA-1 in the intestine under an intestine-specific promoter was sufficient to rescue the calcium defect in *trpa-1* mutant worms, consistent with our genetic data that TRPA-1 can act in the intestine to promote longevity (Figures 6A and 6B).

We then characterized the calcium-impermeable form of TRPA-1 (TRPA-1^{E1018A}) that has lost the capacity to extend lifespan (Figure 3A). Expression of this "calcium-free" TRPA-1 in the intestine failed to restore the cold-evoked calcium response in *trpa-1* mutant worms, providing physiological evidence of a critical role for calcium signaling in lifespan extension at cold temperatures (Figures 6A and 6B). These calcium-imaging data not only complemented our genetic analysis of aging but also revealed a cellular correlate for cold-induced lifespan extension.

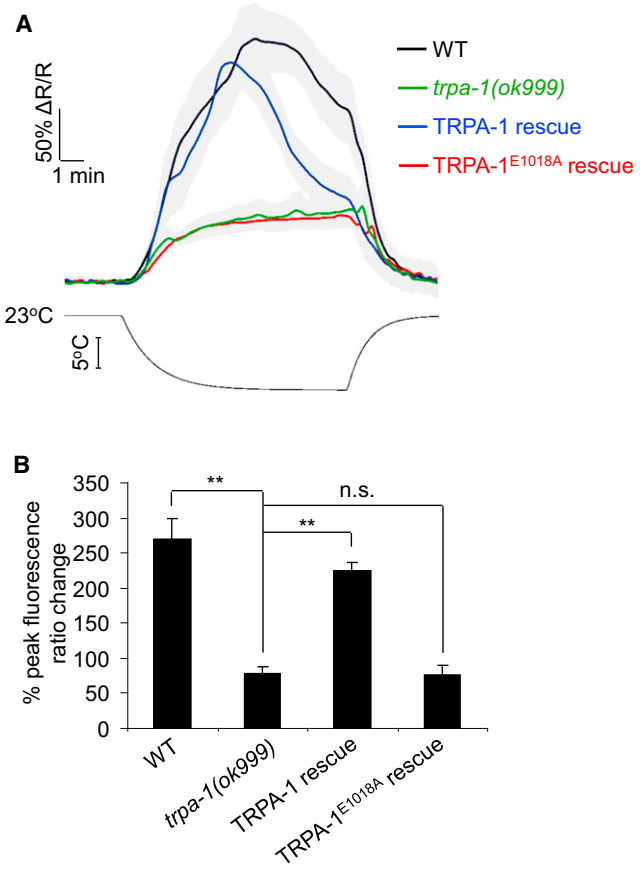


Figure 6. Cold Evokes a Robust Calcium Response in the Intestine

(A) Intestine responds to cooling, and TRPA-1 is important for this response. G-CaMP1.3 was expressed as a transgene in the intestine under the *lfe-2b* promoter (Clandinin et al., 1998). DsRed was coexpressed to enable ratiometric imaging. Shown on the top are the calcium ratio traces. Shades along the traces represent error bars (SEM). Shown on the bottom is the temperature trace.

(B) Bar graph summarizing the data in (A). Error bars: SEM. $n \geq 6$. $^{**}p < 0.0003$ (ANOVA with Bonferroni test).

See also Figure S6 and Table S1.

In addition, they unveiled an unexpected role of intestinal cells as cold receptors in thermosensation, expanding the repertoire of cold receptors from neurons to nonexcitable cells.

Human TRPA1 and a Worm TRPA-1 Variant Can Extend Lifespan in *C. elegans* in a Temperature- and Agonist-Dependent Manner

As human TRPA1 shares a strong homology with worm TRPA-1 (Venkatachalam and Montell, 2007; Xiao and Xu, 2009), we wondered whether it can functionally substitute for its worm homolog in promoting longevity. Remarkably, transgenic expression of human TRPA1 in worms extended lifespan at cold temperatures (Figure 7A) but failed to do so at warm temperatures (Figure 7B). This temperature-dependent effect on lifespan is similar to that observed with worm TRPA-1.

It might be argued that the inability of TRPA channels to extend lifespan at warm temperatures could be due to some nonspecific effects; for example, these channels might not be folded properly or expressed well at high temperatures. To address this concern, it would be necessary to activate these channels at warm temperatures to assess whether they are then able to extend lifespan just like they did at cold temperatures. However, we are unable to do so with worm TRPA-1, as no specific agonists have been found to activate this worm channel. Unlike worm TRPA-1, human TRPA1 can be activated by pungent chemicals, such as AITC (allyl isothiocyanate), which is the active ingredient of wasabi and mustard oil (Bandell et al., 2004; Jordt et al., 2004). The three Cys residues responsible for AITC activation of mammalian TRPA1 somehow are not conserved in worm TRPA-1 (Hinman et al., 2006; Macpherson et al., 2007). Nevertheless, this property of mammalian TRPA1 offers us an opportunity to address the aforementioned concern. We found that, although AITC itself did not notably affect the lifespan of wild-type worms, this agonist extended the lifespan of transgenic worms expressing human TRPA1 at warm temperatures (Figure 7B). Remarkably, this agonist-triggered, human TRPA1-dependent lifespan extension at warm temperatures also depended on PKC-2 and SGK-1, as well as DAF-16 (Figure 7C), suggesting that such lifespan extension is mediated by the same genetic pathway as that recruited by worm TRPA-1 to promote longevity at low temperatures. Thus, though human TRPA1 failed to promote longevity at warm temperatures, it nevertheless was functionally expressed, can be activated with chemical agonists to extend lifespan, and apparently does so by recruiting the same genetic pathway as that employed by worm TRPA-1.

Encouraged by the above results, we engineered a worm TRPA-1 variant, TRPA-1(Cys), by introducing three Cys residues at the same positions as those found in human TRPA1. Although expression of TRPA-1(Cys) extended lifespan at low temperatures (Figure 7D), the same transgene did not promote longevity at warm temperatures (Figure 7E). This is in line with the data obtained with the wild-type form of worm TRPA-1, indicating that the three Cys residues introduced did not globally affect the function of TRPA-1. Interestingly, although TRPA-1(Cys) failed to extend lifespan at warm temperatures, the agonist AITC can promote the lifespan of the same transgenic worms at warm temperatures (Figure 7E). Furthermore, this AITC-triggered lifespan extension at warm temperatures also depended on PKC-2, SGK-1, and DAF-16 (Figure 7F), the same set of genetic components as those recruited by TRPA-1 to extend lifespan at low temperatures. These data indicate that the genetic pathway recruited by TRPA-1 to extend lifespan at low temperatures is a build-in program. In other words, the fact that TRPA-1 cannot extend lifespan at warm temperatures is not because this genetic program is not present or defective at warm temperatures but rather because the cold-sensitive channel TRPA-1 remains closed at warm temperatures. These data, together with the intrinsic temperature sensitivity of these channels reported by others, strongly suggest that the observed temperature dependence of TRPA channels in lifespan regulation is specific. Furthermore, the observation that human TRPA1 can functionally substitute for worm TRPA-1 in lifespan extension also raises the intriguing possibility that the mammalian TRPA1 channel could be a potential target for developing antiaging agents.

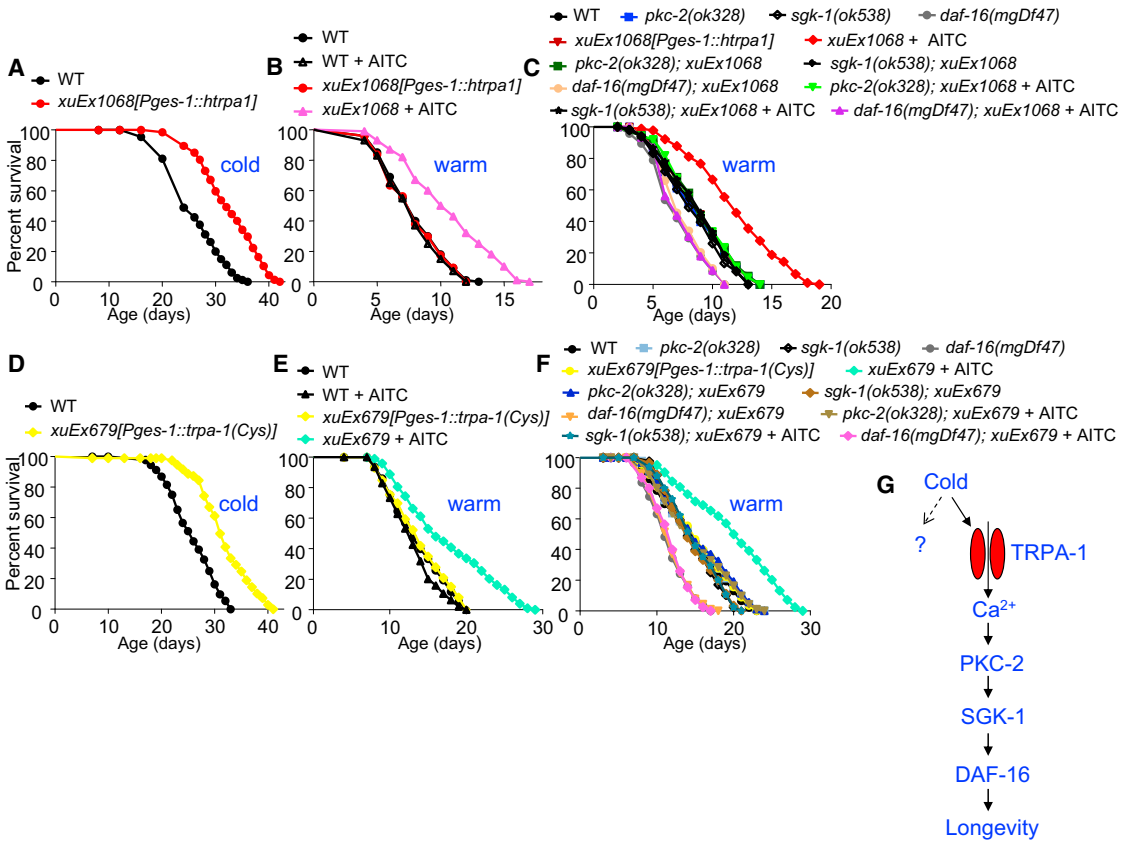


Figure 7. Human TRPA1 and a Worm TRPA-1 Variant Can Extend Lifespan in *C. elegans* in a Temperature- and Agonist-Dependent Manner

(A) Transgenic expression of human TRPA1 (hTRPA1) extends lifespan at cold temperatures. Lifespan was assayed at 15°C.

(B and C) Human TRPA1 (hTRPA1) does not extend lifespan at warm temperatures but can do so in the presence of its agonist AITC (B), and such lifespan extension depends on *pkc-2, sgk-1*, and *daf-16* (C). AITC (10 μM) was included in NGM plates. Lifespan was assayed at 27°C to suppress cold-induced activity of human TRPA1, as this channel is known to have a broad range of activation temperatures (8°C –28°C) (Story et al., 2003).

(D) Transgenic expression of TRPA-1(Cys), a worm TRPA-1 variant incorporated with three Cys residues, extends lifespan at cold temperatures. Lifespan: 15°C.

(E and F) Worm TRPA1(Cys) does not extend lifespan at warm temperatures but can do so in the presence of the agonist AITC (E), and such lifespan extension depends on *pkc-2, sgk-1*, and *daf-16* (F). AITC: 100 μM. Lifespan: 25°C.

(G) A schematic model illustrating a genetic pathway that promotes longevity at cold temperatures in *C. elegans*.

See also Table S1.

DISCUSSION

The past two decades have witnessed a rapid progress in our understanding of how IIS, DR, mitochondria, and germline regulate lifespan (Fontana et al., 2010; Kenyon, 2010). By contrast, very little is known about how temperature reduction promotes longevity. In this study, we have characterized cold-dependent lifespan extension in *C. elegans*. Our results indicate that genetic programs actively contribute to lifespan extension at low temperatures. Apparently, at least in *C. elegans*, the extended lifespan observed at low temperatures cannot be simply explained by a reduced rate of chemical reactions. Thus, cold-dependent lifespan extension is not simply a pure, passive thermodynamic process in *C. elegans*. Notably, lifespan is also regulated at high temperatures (Lee and Kenyon, 2009). Given that the mechanisms regulating lifespan show striking conservation across phylogeny (Fontana et al., 2010; Kenyon, 2010), our work raises the possibility that a similar phenomenon may also occur in other organisms.

TRP Channels and Calcium Signaling in Lifespan Regulation

Our data suggest a model in which low temperatures activate the cold-sensitive TRPA-1 channel, and the ensuing calcium influx via TRPA-1 then stimulates the calcium-sensitive PKC-2, which signals via SGK-1 to DAF-16/FOXO, leading to an increase in longevity (Figure 7G). It should be noted that this model proposes a genetic rather than biochemical pathway through which cold temperatures regulate lifespan. Future studies are needed to elucidate the detailed biochemical mechanisms. Notably, our model links calcium signaling to longevity. Though calcium is well known as a key second messenger implicated in a wide range of physiological processes, surprisingly, a direct role of calcium signaling in regulating lifespan has not been

appreciated. As numerous neurotransmitters, neuropeptides, and hormones as well as many signaling pathways modulate calcium dynamics, it would be interesting to investigate whether they also regulate lifespan.

Our results suggest that TRPA-1 acts a thermosensor to detect temperature drop in the environment to initiate a pro-longevity genetic program. This uncovers a previously unrecognized function for TRP-family channels. Expression of TRPA-1 in the intestine or neurons is sufficient to promote lifespan, consistent with the view that the intestine and nervous system are the two key tissues in lifespan regulation (Kenyon, 2010). Interestingly, human TRPA1 can functionally substitute for its worm homolog in lifespan extension. Similar to worm TRPA-1, mammalian TRPA1 is also expressed in neurons as well as several nonexcitable tissues (Earley et al., 2009; Stokes et al., 2006). It will be interesting to test whether this channel regulates lifespan in mammals. As feeding the TRPA1 agonist AITC to transgenic worms expressing human TRPA1 can extend lifespan, one simple experiment would be to test whether AITC or other TRPA1 agonists can extend lifespan in mice.

Nevertheless, our studies do not exclude the contribution of additional mechanisms to lifespan extension at low temperatures. *trpa-1* mutant worms, though short lived at low temperatures, exhibit a lifespan comparable to that of wild-type worms at warm temperatures, suggesting that additional mechanisms must contribute. For example, some yet-to-be identified cold-sensitive proteins may function in parallel to TRPA-1. In addition, the classic mechanism, i.e., a reduced rate of chemical reactions at low temperatures, may also contribute. Future studies are needed to uncover other unknown genetic programs and identify additional components in the TRPA-1 pathway, which ultimately may lead to a thorough understanding of how cold temperatures promote longevity in *C. elegans*.

The Intestine, a Signaling Hub in Lifespan Regulation, Is a Cold Receptor

We showed that cold evokes a robust TRPA-1-dependent calcium response in the intestine, providing a cellular correlate for cold-induced lifespan extension. This result also identifies the intestine as a cold receptor, uncovering an unexpected role for the intestine in thermosensation. Although neurons are well known to act as cold receptors in various organisms (Chatzigeorgiou et al., 2010; Gallio et al., 2011; Story et al., 2003), no nonexcitable cells have been reported to respond to cold. This finding thus expands the repertoire of cold receptors from neurons to nonexcitable cells. Though both the nervous system and intestine are considered as key tissues in lifespan regulation, the intestine plays a special role in that it integrates multiple pro- and antiaging cues and also signals to other tissues to coordinate a body-wide response (Kenyon, 2010; Libina et al., 2003). The finding that the intestine responds to cold highlights the role of this tissue as a signaling hub in lifespan regulation.

It is worth noting that the intestine is also the fat tissue of *C. elegans* (Soukas et al., 2009). Interestingly, the fat tissue also plays an important role in lifespan control in mammals (Blüher et al., 2003), underscoring a general role of the fat/intestine tissue in aging. In mammals, both the white and brown fat tissues are distributed right underneath the skin and may undergo some level of fluctuations in temperature when exposed to cold environments, though the core body temperature likely remains constant. It is conceivable that such temperature fluctuations in the fat tissue may trigger aging-related signaling events. A similar phenomenon may occur in the sensory neurons with their nerve endings embedded in the skin, which can certainly sense temperature drop in the environment. Currently, lowering the whole body temperature of homeothermic animals, such as mouse, via transgenic approaches is known to extend lifespan (Conti et al., 2006); however, whether a simple cooling of their peripheral tissues (e.g., skin and fat) by exposing these animals to cold environments affects lifespan has not been examined. Our study may encourage researchers to explore this intriguing possibility.

EXPERIMENTAL PROCEDURES

Strains

Wild-type: N2. TQ1516: *trpa-1(ok999)* ×6 outcrossed. TQ2571: *pkc-2(ok328)* ×6 outcrossed. TQ1697: *xuEx619[trpa-1(genomic) + sur-5::gfp]*. TQ1643: *xuEx601[Pges-1::trpa-1::SL2::yfp + Punc-122::DsRed]*. TQ1648: *xuEx606 [Prgef-1::trpa-1::SL2::yfp + Punc-122::DsRed]*. TQ1657: *xuEx610[Pmyo-3::trpa-1::SL2::yfp + Punc-122::DsRed]*. TQ1658: *xuEx611[Pdpy-7::trpa-1::SL2::yfp + Punc-122::DsRed]*. TQ1654: *daf-16(mgDf47)*. TQ2014: *daf-16(mgDF47); xuEx601*. TJ356: *zIs356[daf-16::gfp + roller]*. TQ2505: *zIs356; xuEx785*. TQ1939: *xuEx676[Pges-1::trpa-1(E1018A)::SL2::yfp + Punc-122::DsRed]*. TQ2674: *pkc-2(ok328);xuEx601*. TQ2230: *pkc-2(ok328); xuEx619*. TQ2789: *xuEx913[Pges-1::pkc-2::SL2::mCherry]*. TQ2845: *daf-16(mgDf47); xuEx913*. TQ2853: *zIs356; xuEx911[Pges-1::pkc-2::SL2::mCherry]*. BQ1: *akt-1(mg306)*. TQ2212: *akt-1(mg306); xuEx601*. TQ2927: *akt-2(ok393)* ×4 outcrossed. TQ2928: *akt-2(ok391); xuEx601*. TQ2746: *cmk-1(oy21)* ×4 outcrossed. TQ2749: *cmk-1(oy21); xuEx601*. TQ2752: *cmk-1(oy21); xuEx619*. TQ1996: *unc-43(n498,n1186)* ×4 outcrossed. TQ2351: *unc-43(n498,n1186); xuEx601*. All RNAi clones were from the Ahringer library and confirmed by sequencing.

Lifespan Assay

Unless indicated otherwise, all lifespan studies were conducted at 20°C (Hsu et al., 2009). In all experiments, the first day of adulthood was scored as day 1. For each lifespan assay, 80–100 worms were included and transferred every 2–3 days to fresh 60 mm NGM (nematode growth medium) plates at a density of 10 worms per plate. Worms were censored if they crawled off the plate, exploded, or bagged. FUdR was included in assays involving *sgk-1* mutant worms, which show a defect in egg laying. For RNAi experiments, NGM plates were supplemented with carbenicillin (25 μg/ml) and IPTG (1 mM). HT115 bacteria containing either empty vector L4440 or RNAi plasmid were seeded on RNAi plates 2 days before experiments. All statistical analyses were performed with GraphPad Prism 5 (GraphPad Software, Inc.) and IBM SPSS Statistics 19 (IBM, Inc.). p values were calculated using the log-rank (Kaplan-Meier) method.

Calcium Imaging

Calcium imaging was performed on an Olympus upright microscope (BX51WI) under a 60× objective with a protocol described previously (Feng et al., 2006; Li et al., 2006). Worms were glued on an agarose pad and incubated in bath solution: 10 mM HEPES (pH 7.4), 5 mM KCl, 145 mM NaCl, 1.2 mM $MgCl_2$, 2.5 mM $CaC1_2$, and 10 mM glucose. Bath solution of varying temperatures was perfused toward the animal. Its temperature was controlled by a bipolar in-line temperature controller (Warner Instruments) triggered by an EPC10 amplifier. Images were acquired with a Roper CoolSnap CCD camera and processed by MetaFluor (Molecular Devices, Inc.). G-CaMP and DsRed fluorescence were excited at 484 nm and 535 nm, respectively, and peak percentage change in the ratio of G-CaMP/DsRed fluorescence was quantified.

qRT-PCR

Total RNA was extracted with TRI Reagent (Life Technologies) from ~150 worms. The relative amount of mRNA of *daf-16* target genes was analyzed by quantitative PCR (qPCR) with CYBR Green (Life Technologies) according the protocol provided by the manufacturer. *act-1* (actin) was used as an internal reference for normalization. ΔΔCt method was used to analyze the qPCR data.

SUPPLEMENTAL INFORMATION

Supplemental Information includes six figures and one table and can be found with this article online at http://dx.doi.org/10.1016/j.cell.2013.01.020.

ACKNOWLEDGMENTS

We thank Ao-Lin Hsu for providing reagents and critically reading the manuscript, Jiandie Lin, Xiaowei Chen, and Zhaoyu Li for technical assistance, and Sean Curran and Gary Ruvkun for *daf-16* strains. Some strains were obtained from the CGC and Knockout Consortiums in the US and Japan. R.X. was supported by an AHA postdoctoral fellowship. This work was supported by the Program of Introducing Talents of Discipline to Universities from the Ministry of Education of China (J.L.) and grants from the NIGMS (X.Z.S.X.), NIA (Ao-Lin Hsu), NSFC (T.X. and X.Z.S.X.), and Pew scholar program (X.Z.S.X.).

Received: July 1, 2012
Revised: October 23, 2012
Accepted: January 11, 2013
Published: February 14, 2013

REFERENCES

Aamodt, E.J., Chung, M.A., and McGhee, J.D. (1991). Spatial control of gut-specific gene expression during Caenorhabditis elegans development. Science *252*, 579–582.

Alam, H., Williams, T.W., Dumas, K.J., Guo, C., Yoshina, S., Mitani, S., and Hu, P.J. (2010). EAK-7 controls development and life span by regulating nuclear DAF-16/FoxO activity. Cell Metab. *12*, 30–41.

Altun-Gultekin, Z., Andachi, Y., Tsalik, E.L., Pilgrim, D., Kohara, Y., and Hobert, O. (2001). A regulatory cascade of three homeobox genes, ceh-10, ttx-3 and ceh-23, controls cell fate specification of a defined interneuron class in C. elegans. Development *128*, 1951–1969.

Antebi, A., Yeh, W.H., Tait, D., Hedgecock, E.M., and Riddle, D.L. (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., and Kenyon, C. (1999). Regulation of lifespan by sensory perception in Caenorhabditis elegans. Nature *402*, 804–809.

Bandell, M., Story, G.M., Hwang, S.W., Viswanath, V., Eid, S.R., Petrus, M.J., Earley, T.J., and Patapoutian, A. (2004). Noxious cold ion channel TRPA1 is activated by pungent compounds and bradykinin. Neuron *41*, 849–857.

Bishop, N.A., and Guarente, L. (2007). Two neurons mediate diet-restriction-induced longevity in C. elegans. Nature *447*, 545–549.

Blüher, M., Kahn, B.B., and Kahn, C.R. (2003). Extended longevity in mice lacking the insulin receptor in adipose tissue. Science *299*, 572–574.

Chatzigeorgiou, M., Yoo, S., Watson, J.D., Lee, W.H., Spencer, W.C., Kindt, K.S., Hwang, S.W., Miller, D.M., 3rd, Treinin, M., Driscoll, M., and Schafer, W.R. (2010). Specific roles for DEG/ENaC and TRP channels in touch and thermosensation in C. elegans nociceptors. Nat. Neurosci. *13*, 861–868.

Clandinin, T.R., DeModena, J.A., and Sternberg, P.W. (1998). Inositol trisphosphate mediates a RAS-independent response to LET-23 receptor tyrosine kinase activation in *C. elegans*. Cell *92*, 523–533.

Conti, B. (2008). Considerations on temperature, longevity and aging. Cell. Mol. Life Sci. *65*, 1626–1630.

Conti, B., Sanchez-Alavez, M., Winsky-Sommerer, R., Morale, M.C., Lucero, J., Brownell, S., Fabre, V., Huitron-Resendiz, S., Henriksen, S., Zorrilla, E.P., et al. (2006). Transgenic mice with a reduced core body temperature have an increased life span. Science *314*, 825–828.

Dupuy, D., Bertin, N., Hidalgo, C.A., Venkatesan, K., Tu, D., Lee, D., Rosenberg, J., Svrzikapa, N., Blanc, A., Carnec, A., et al. (2007). Genome-scale analysis of in vivo spatiotemporal promoter activity in Caenorhabditis elegans. Nat. Biotechnol. *25*, 663–668.

Earley, S., Gonzales, A.L., and Crnich, R. (2009). Endothelium-dependent cerebral artery dilation mediated by TRPA1 and Ca2+-Activated K+ channels. Circ. Res. *104*, 987–994.

Feng, Z., Li, W., Ward, A., Piggott, B.J., Larkspur, E.R., Sternberg, P.W., and Xu, X.Z.S. (2006). A *C. elegans* model of nicotine-dependent behavior: regulation by TRP-family channels. Cell *127*, 621–633.

Fire, A., and Waterston, R.H. (1989). Proper expression of myosin genes in transgenic nematodes. EMBO J. *8*, 3419–3428.

Fontana, L., Partridge, L., and Longo, V.D. (2010). Extending healthy life span—from yeast to humans. Science *328*, 321–326.

Gallio, M., Ofstad, T.A., Macpherson, L.J., Wang, J.W., and Zuker, C.S. (2011). The coding of temperature in the Drosophila brain. Cell *144*, 614–624.

Gilleard, J.S., Barry, J.D., and Johnstone, I.L. (1997). cis regulatory requirements for hypodermal cell-specific expression of the Caenorhabditis elegans cuticle collagen gene dpy-7. Mol. Cell. Biol. *17*, 2301–2311.

Henderson, S.T., and Johnson, T.E. (2001). daf-16 integrates developmental and environmental inputs to mediate aging in the nematode *Caenorhabditis elegans*. Curr. Biol. *11*, 1975–1980.

Hertweck, M., Göbel, C., and 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.

Hinman, A., Chuang, H.H., Bautista, D.M., and Julius, D. (2006). TRP channel activation by reversible covalent modification. Proc. Natl. Acad. Sci. USA *103*, 19564–19568.

Hsu, A.L., Feng, Z., Hsieh, M.Y., and Xu, X.Z. (2009). Identification by machine vision of the rate of motor activity decline as a lifespan predictor in C. elegans. Neurobiol. Aging *30*, 1498–1503.

Hsu, A.L., Murphy, C.T., and Kenyon, C. (2003). Regulation of aging and age-related disease by DAF-16 and heat-shock factor. Science *300*, 1142–1145.

Islas-Trejo, A., Land, M., Tcherepanova, I., Freedman, J.H., and Rubin, C.S. (1997). Structure and expression of the Caenorhabditis elegans protein kinase C2 gene. Origins and regulated expression of a family of Ca2+-activated protein kinase C isoforms. J. Biol. Chem. *272*, 6629–6640.

Jia, K., Albert, P.S., and Riddle, D.L. (2002). DAF-9, a cytochrome P450 regulating C. elegans larval development and adult longevity. Development *129*, 221–231.

Jordt, S.E., Bautista, D.M., Chuang, H.H., McKemy, D.D., Zygmunt, P.M., Högestätt, E.D., Meng, I.D., and Julius, D. (2004). Mustard oils and cannabinoids excite sensory nerve fibres through the TRP channel ANKTM1. Nature *427*, 260–265.

Karashima, Y., Talavera, K., Everaerts, W., Janssens, A., Kwan, K.Y., Vennekens, R., Nilius, B., and Voets, T. (2009). TRPA1 acts as a cold sensor in vitro and in vivo. Proc. Natl. Acad. Sci. USA *106*, 1273–1278.

Kenyon, C.J. (2010). The genetics of ageing. Nature *464*, 504–512.

Kimura, K.D., Tissenbaum, H.A., Liu, Y., and Ruvkun, G. (1997). daf-2, an insulin receptor-like gene that regulates longevity and diapause in Caenorhabditis elegans. Science *277*, 942–946.

Kindt, K.S., Viswanath, V., Macpherson, L., Quast, K., Hu, H., Patapoutian, A., and Schafer, W.R. (2007). Caenorhabditis elegans TRPA-1 functions in mechanosensation. Nat. Neurosci. *10*, 568–577.

Klass, M.R. (1977). Aging in the nematode Caenorhabditis elegans: major biological and environmental factors influencing life span. Mech. Ageing Dev. *6*, 413–429.

Lee, B.H., and Ashrafi, K. (2008). A TRPV channel modulates C. elegans neurosecretion, larval starvation survival, and adult lifespan. PLoS Genet. *4*, e1000213.

Lee, S.J., and Kenyon, C. (2009). Regulation of the longevity response to temperature by thermosensory neurons in *Caenorhabditis elegans*. Curr. Biol. *19*, 715–722.

Lee, S.S., Kennedy, S., Tolonen, A.C., and Ruvkun, G. (2003). DAF-16 target genes that control C. elegans life-span and metabolism. Science *300*, 644–647.

Li, J., Ebata, A., Dong, Y., Rizki, G., Iwata, T., and Lee, S.S. (2008). Caenorhabditis elegans HCF-1 functions in longevity maintenance as a DAF-16 regulator. PLoS Biol. *6*, e233.

Li, W., Feng, Z., Sternberg, P.W., and Xu, X.Z.S. (2006). A C. elegans stretch receptor neuron revealed by a mechanosensitive TRP channel homologue. Nature *440*, 684–687.

Libina, N., Berman, J.R., and Kenyon, C. (2003). Tissue-specific activities of *C. elegans* DAF-16 in the regulation of lifespan. Cell *115*, 489–502.

Lin, K., Dorman, J.B., Rodan, A., and 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., and Kenyon, C. (2001). Regulation of the Caenorhabditis elegans longevity protein DAF-16 by insulin/IGF-1 and germline signaling. Nat. Genet. *28*, 139–145.

Loeb, J., and Northrop, J.H. (1916). Is there a temperature coefficient for the duration of life? Proc. Natl. Acad. Sci. USA *2*, 456–457.

Macpherson, L.J., Dubin, A.E., Evans, M.J., Marr, F., Schultz, P.G., Cravatt, B.F., and Patapoutian, A. (2007). Noxious compounds activate TRPA1 ion channels through covalent modification of cysteines. Nature *445*, 541–545.

McKemy, D.D., Neuhausser, W.M., and Julius, D. (2002). Identification of a cold receptor reveals a general role for TRP channels in thermosensation. Nature *416*, 52–58.

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.

Nakai, J., Ohkura, M., and Imoto, K. (2001). A high signal-to-noise Ca(2+) probe composed of a single green fluorescent protein. Nat. Biotechnol. *19*, 137–141.

Ogg, S., Paradis, S., Gottlieb, S., Patterson, G.I., Lee, L., Tissenbaum, H.A., and 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, S.W., Mukhopadhyay, A., Svrzikapa, N., Jiang, F., Davis, R.J., and Tissenbaum, H.A. (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, S.W., Mukhopadhyay, A., Dixit, B.L., Raha, T., Green, M.R., and Tissenbaum, H.A. (2006). Identification of direct DAF-16 targets controlling longevity, metabolism and diapause by chromatin immunoprecipitation. Nat. Genet. *38*, 251–257.

Panowski, S.H., Wolff, S., Aguilaniu, H., Durieux, J., and Dillin, A. (2007). PHA-4/Foxa mediates diet-restriction-induced longevity of C. elegans. Nature *447*, 550–555.

Paradis, S., and 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.

Peier, A.M., Moqrich, A., Hergarden, A.C., Reeve, A.J., Andersson, D.A., Story, G.M., Earley, T.J., Dragoni, I., McIntyre, P., Bevan, S., and Patapoutian, A. (2002). A TRP channel that senses cold stimuli and menthol. Cell *108*, 705–715.

Reiner, D.J., Newton, E.M., Tian, H., and Thomas, J.H. (1999). Diverse behavioural defects caused by mutations in Caenorhabditis elegans unc-43 CaM kinase II. Nature *402*, 199–203.

Satterlee, J.S., Ryu, W.S., and Sengupta, P. (2004). The CMK-1 CaMKI and the TAX-4 Cyclic nucleotide-gated channel regulate thermosensory neuron gene expression and function in *C. elegans*. Curr. Biol. *14*, 62–68.

Soukas, A.A., Kane, E.A., Carr, C.E., Melo, J.A., and Ruvkun, G. (2009). Rictor/TORC2 regulates fat metabolism, feeding, growth, and life span in Caenorhabditis elegans. Genes Dev. *23*, 496–511.

Stokes, A., Wakano, C., Koblan-Huberson, M., Adra, C.N., Fleig, A., and Turner, H. (2006). TRPA1 is a substrate for de-ubiquitination by the tumor suppressor CYLD. Cell. Signal. *18*, 1584–1594.

Story, G.M., Peier, A.M., Reeve, A.J., Eid, S.R., Mosbacher, J., Hricik, T.R., Earley, T.J., Hergarden, A.C., Andersson, D.A., Hwang, S.W., et al. (2003). ANKTM1, a TRP-like channel expressed in nociceptive neurons, is activated by cold temperatures. Cell *112*, 819–829.

Tullet, J.M., Hertweck, M., An, J.H., Baker, J., Hwang, J.Y., Liu, S., Oliveira, R.P., Baumeister, R., and Blackwell, T.K. (2008). Direct inhibition of the longevity-promoting factor SKN-1 by insulin-like signaling in C. elegans. Cell *132*, 1025–1038.

Venkatachalam, K., and Montell, C. (2007). TRP channels. Annu. Rev. Biochem. *76*, 387–417.

Wang, Y.Y., Chang, R.B., Waters, H.N., McKemy, D.D., and Liman, E.R. (2008). The nociceptor ion channel TRPA1 is potentiated and inactivated by permeating calcium ions. J. Biol. Chem. *283*, 32691–32703.

Wolff, S., Ma, H., Burch, D., Maciel, G.A., Hunter, T., and Dillin, A. (2006). SMK-1, an essential regulator of DAF-16-mediated longevity. Cell *124*, 1039–1053.

Wu, D., Rea, S.L., Cypser, J.R., and Johnson, T.E. (2009). Mortality shifts in Caenorhabditis elegans: remembrance of conditions past. Aging Cell *8*, 666–675.

Xiao, R., and Xu, X.Z.S. (2009). Function and regulation of TRP family channels in C. elegans. Pflugers Arch. *458*, 851–860.