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## *C. elegans* Aging: Proteolysis Cuts Both Ways

Recent reports from two laboratories working on the nematode *Caenorhabditis elegans* have identified both positive and negative roles for ubiquitin-mediated proteolysis in the regulation of longevity by the insulin/insulin-like growth factor signaling pathway.

### Bruce Bowerman

Over the past 20 years, pioneering genetic studies in the roundworm *Caenorhabditis elegans* have established that aging is genetically programmed [1–4]. This helps to explain how even closely related animals can exhibit remarkably different lifespans. For example, one rodent, the rat, sneaks around for only about 3 years, while squirrels can dash about for 25.

The best understood longevity regulator, conserved from roundworms to mammals, is the insulin/insulin-like growth factor signaling (IIS) pathway (Figure 1) [3,4]. The single IIS receptor in *C. elegans*, called DAF-2, activates downstream kinases that phosphorylate and thereby oppose the nuclear localization of DAF-16, a FOXO transcription factor. Loss of IIS reduces this phosphorylation and DAF-16 transits to the nucleus, where it activates some genes and represses others to extend lifespan. By contrast, loss of DAF-16/FOXO decreases lifespan, both in otherwise wild-type worms and in mutants lacking IIS. Thus, IIS limits lifespan by altering the expression of genes that influence longevity. However, additional upstream inputs also influence DAF-16 [5]. Recently published studies from two groups now show that ubiquitin-mediated proteolysis accounts for at least some of this additional input, and

that proteolytic regulation can both oppose and promote longevity [6,7]. These studies provide the first evidence that ubiquitin-mediated proteolysis influences lifespan, adding one more target to the ever-growing list of proteasome-regulated processes.

The discovery that genetic programs control lifespan in *C. elegans* began in large part with studies of dauer formation, a developmental switch that produces long-lived larvae to endure tough times. To investigate this resilient lifestyle, geneticists identified large numbers of dauer formation-defective and dauer formation-constitutive (Daf-d and Daf-c) mutants [8]. The mutated genes were ordered into a regulatory pathway and some, including DAF-2, were found to encode IIS pathway components [3,4,8,9]. One property of many dauer pathway genes is that even null alleles are temperature sensitive: indeed, dauer formation is a temperature-sensitive process that is favored at higher temperatures.

While dauer larvae are impressively long-lived, the DAF connection to aging was not made until 1993, when Cynthia Kenyon and colleagues [2] raised *daf-2* mutants at a lower permissive temperature until they became adults. They found that adult *daf-2(-)* mutants proved to be remarkably long-lived, while adult

*daf-16(-)* and *daf-2(-); daf-16(-)* mutants were remarkably short-lived. Thus, as in dauer formation, IIS acts through DAF-16/FOXO to limit lifespan. However, the effects on lifespan could be temporally uncoupled from the requirements for dauer formation. Subsequent to this discovery, the contributions of IIS and other inputs to aging in *C. elegans* have been studied extensively, and IIS has been shown to influence longevity in a variety of animals [4].

Now Li *et al.* [6] report that DAF-16/FOXO is targeted for degradation by poly-ubiquitination, after using *C. elegans* to study a murine E3 ubiquitin ligase called Roquin that functions in autoimmunity [10]. Using a transposon-tagged allele of *rle-1*, the *C. elegans* ortholog of Roquin, Li *et al.* [6] found that *rle-1* mutants are long-lived, and that this lifespan extension requires DAF-16 (hence the gene name, for regulation of longevity). Disruption of *rle-1* elevates DAF-16/FOXO protein levels and increases the transcription of DAF-16 target genes that extend lifespan. Furthermore, RLE-1 binds to DAF-16 and promotes its polyubiquitination and proteasome-dependent degradation. Finally, the transposon-tagged *rle-1* allele produces a truncated RLE-1 protein that fails to bind DAF-16 *in vitro*. These findings all suggest that RLE-1 opposes longevity by targeting DAF-16/FOXO for degradation. Thus, proteolytic regulation, in addition to IIS-dependent phosphorylation, limits lifespan by negatively regulating DAF-16. Intriguingly, the authors also showed that *rle-1(-); daf-16(-)* double mutants live longer than *daf-16(-)* single

mutants, suggesting that RLE-1 has targets in addition to DAF-16. As the amino-terminal domains in RLE-1 might influence other factors, it is possible that a definitive *rle-1* null allele would further extend lifespan.

While DAF-16 clearly plays an important role, other factors downstream of IIS also appear to influence longevity. For example, a constitutively nuclear form of DAF-16/FOXO extends lifespan more in *daf-2(-); daf-16(-)* mutants than it does in *daf-16(-)* mutants [5]. Thus, in addition to preventing DAF-16 nuclear localization, IIS apparently downregulates other longevity-promoting factors. Recently published work from the Kenyon lab [7] suggests that ubiquitin-mediated proteolysis influences these other as yet unknown factors.

In contrast to the fortuitous but revealing findings reported by Li *et al.* [6], Ghazi *et al.* [7] used a targeted and systematic genetic screen to identify proteolytic regulators of longevity. An earlier genome-wide RNAi screen for regulators of longevity indicated that some proteolytic factors influence lifespan [11]. Ghazi *et al.* focused specifically on the proteasome and found that RNAi-mediated depletion of its function shortened lifespan not only in wild-type worms but also in long-lived mutants. They then examined requirements for the cullin-based E3 ligases that target proteins for polyubiquitination and subsequent degradation by the proteasome. They found that depleting some of the *C. elegans* cullin scaffolding proteins reduced the extension of lifespan in IIS mutants, but not in wild-type worms, or in worms with increased lifespan caused by either reduction of the germline or caloric restriction. Depletion of the cullin 1 family member (CUL-1) was most effective at specifically reducing IIS mutant lifespan. Ghazi *et al.* concluded that the cullin-1-based SCF class of E3 ubiquitin ligases targets some proteins to the proteasome to specifically influence the regulation of longevity by IIS.

SCF E3 ligases use Skp1p-related and F-box proteins as adaptors to recruit substrates for

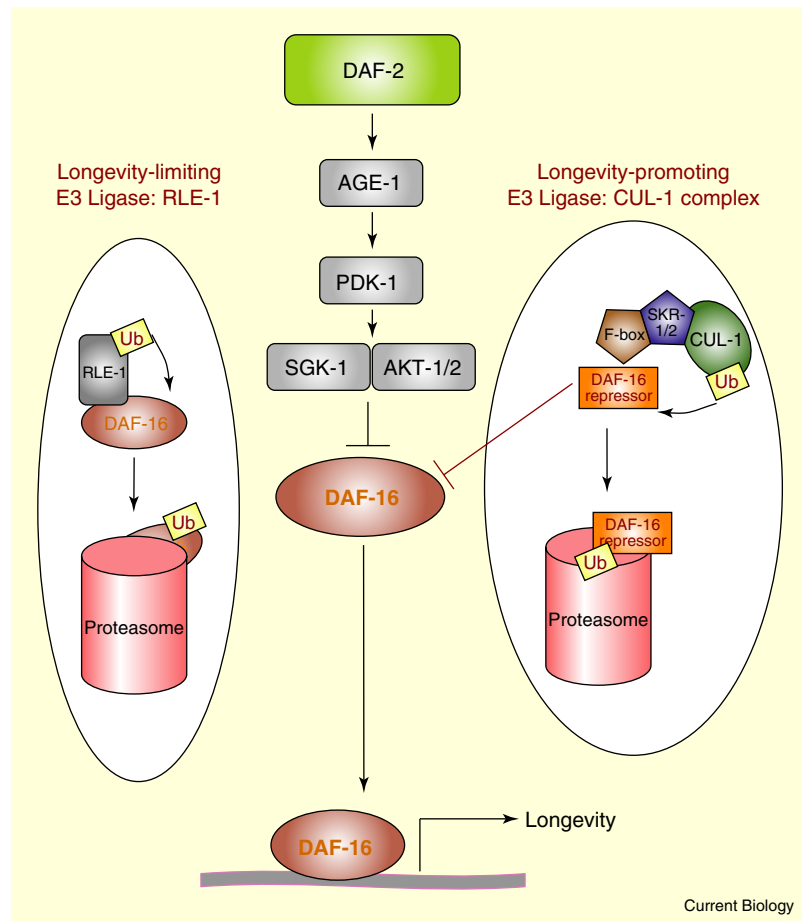


Figure 1. The insulin/insulin-like growth factor signaling (IIS) pathway and ubiquitin-mediated proteolysis regulate longevity in *C. elegans*.

The FOXO transcription factor DAF-16 regulates the expression of target genes in the nucleus to promote longevity. The IIS receptor DAF-2 acts through a cascade of kinases to limit longevity by inhibiting the nuclear translocation of DAF-16. DAF-16 and longevity also are downregulated by RLE-1, an E3 ligase that promotes the polyubiquitination and subsequent degradation of DAF-16 by the proteasome (on the left). SCF E3 ligases appear to target for degradation other factors that also somehow oppose DAF-16 and its effect on longevity (on the right). Thus the SCF E3 ligases promote longevity, by targeting as yet unidentified negative regulators of DAF-16 to the proteasome. See the text for details and references. (Figure courtesy of Arjumand Ghazi; thanks to both Arjumand Ghazi and Cynthia Kenyon for comments on this manuscript.)

polyubiquitination and subsequent degradation [12]. Ghazi *et al.* [7] therefore depleted Skp1p-related proteins in *C. elegans* and identified two, SKR-1 and SKR-2, that are 83% identical in amino acid sequence and exhibit the greatest requirement for lifespan extension in IIS mutant worms. As with CUL-1, depletion of SKR-1/2 did not reduce lifespan in wild-type worms or in mutants with a reduced germline. The *C. elegans* genome includes a daunting 500+ genes that encode F-box proteins. Ghazi *et al.* identified four that reduce lifespan in long-lived mutants but not in wild-type

worms. Of these, one called LIN-23 has been shown previously to encode an SCF E3 ligase adaptor that targets cell-cycle progression [13]. However, a *lin-23* mutation that does not disrupt cell-cycle regulation [14] does reduce lifespan in IIS mutants, suggesting that the lifespan influence is cell cycle independent. Only one of the four F-box genes, called PHI-3, was specific for IIS mutants, while depletion of the other three reduced lifespan to varying degrees in other long-lived mutants. Finally, these SCF components do not act directly through DAF-16: their depletion did

not reduce DAF-16 levels or nuclear localization and still reduced lifespan in worms expressing a constitutively nuclear form of DAF-16. Nevertheless, depletion of two SCF E3 ligase F-box components (LIN-23 and PHI-3) did prevent the increased expression of genes activated by DAF-16, suggesting that they target cofactors that affect the ability of DAF-16 to activate longevity-promoting genes.

The results from Li *et al.* [6] and Ghazi *et al.* [7] show that ubiquitin-mediated proteolytic regulation targets both DAF-16/FOXO to limit lifespan and additional factors to extend lifespan. Moreover, it was recently shown that inactivation of a *C. elegans* F-box protein called DRE-1 results in the precocious expression of some cell fates expressed during larval development [15]. Larval transitions in *C. elegans* are temporally regulated and reminiscent of aging but occur earlier in life. Intriguingly, CUL-1 in vertebrates targets FOXO family members for degradation [16], and it will of course be interesting to learn whether ubiquitin-mediated proteolysis influences lifespan and aging in other animals.

In closing, the regulation of longevity is complex and multifaceted, and much remains unknown [4]. For example, reducing IIS doubles lifespan,

while removing the germline in *C. elegans* IIS mutants results in a remarkable sixfold increase in lifespan [17]. In addition, mitochondrial function influences longevity, and the NAD-dependent histone deacetylase Sir2 also extends lifespan [4]. Each animal genome includes hundreds of genes that encode the machinery of proteolysis; exactly how many of them regulate lifespan remains to be seen.

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## Host Genetics: Fine-Tuning Innate Signaling

A polymorphism modulating innate immunity signal transduction has recently been shown to influence human susceptibility to many different infections, providing one more indication of the potential of host genetics to reveal physiological pathways and mechanisms that influence resistance to infectious diseases.

Jacques Fellay  
and David B. Goldstein

A striking feature of all known infectious diseases is that no matter how devastating they may be there is always variation among humans in their susceptibility. In

the middle of the 14<sup>th</sup> century, some people inexplicably survived Black Death while their entire villages were devastated [1]; in African cities with a high prevalence of HIV/AIDS, certain prostitutes remain uninfected after years of unprotected sexual

contact [2]; more prosaically, some of us seem resistant to the flu virus without ever having had a vaccination.

A few of the underlying genetic causes for this variation have been shown to affect elements of both innate and adaptive immunity. Recent work has now established solid evidence for two hypotheses long suspected but as yet with little empirical evidence: first, that there are intermediate levels of immune response that are optimal, and second, that some genetic differences confer advantages against a broad range of infectious agents [3].