A Conserved Regulatory System for Aging

Minireview

Cynthia Kenyon*
Department of Biochemistry and Biophysics
University of California, San Francisco
San Francisco, California 94143

While many processes in biology, such as cell differentiation and development, increase complexity, the aging process increases entropy and culminates in the death of the animal. Thus, the discovery that single gene mutations in many organisms can extend lifespan dramatically was surprising. These mutations indicated that the aging process is subject to regulation; it is not as random and haphazard as it seems. Even more surprising are some recent findings suggesting that a conserved system regulating lifespan may have arisen early in evolution. This regulatory system also controls diapause-like states, which are relatively quiescent states that allow an animal to postpone reproduction in response to adverse environmental conditions. This connection is particularly intriguing because, in at least some cases, lifespan extension can be uncoupled from other aspects of diapause, allowing active and fertile animals to remain youthful much longer than normal.

Insulin/IGF-1 Signaling in C. elegans

A pathway that regulates both lifespan and diapause was first discovered in *C. elegans* (reviewed in Guarente and Kenyon, 2000). In response to food limitation and crowding, juvenile worms enter a state of diapause, called dauer. Dauers are developmentally arrested, reproductively immature, resistant to oxidative stress, and long-lived. As they enter the dauer state, the animals synthesize food-storage substances such as fat, which they metabolize as dauers. When conditions improve, they become fertile adults with normal lifespans (Figure 1). Replete environments stimulate growth to adulthood by activating an insulin/IGF-1 signaling pathway. Loss-of-function mutations in the insulin/IGF-1 receptor homolog *daf-2*, or in downstream components of a conserved PI3-kinase/PDK/Akt pathway cause dauer formation even when food is present. This pathway appears to act by downregulating a forkhead/winged-helix transcription factor called DAF-16 (Figure 2). In the absence of DAF-16, neither food limitation nor insulin/IGF-1 pathway mutations induce dauer formation.

Weak mutations in the insulin/IGF-1 pathway allow the animals to grow to adulthood, where they remain youthful much longer than normal, and live more than twice as long. Animals carrying different insulin/IGF-1 pathway mutations differ in the extent to which they express other dauer-like traits (see Guarente and Kenyon, 2000). Certain mutations cause adults to reproduce late in life (or not at all), to lie still, adopting a curved, dauer-like posture, and/or to synthesize high levels of fat. Like dauers, some mutant adults have a low metabolic rate, and all mutants tested exhibit resistance to

oxidative stress. However, remarkably, most of these dauer-like traits can be uncoupled from lifespan extension. It is possible to obtain long-lived mutant strains that move normally, are fully fertile, and have a normal metabolic rate. In addition, it has also been possible to obtain long-lived genetic mosaics that do not produce fat. The fact that these long-lived mutants can appear so normal makes one optimistic that if this system exists in humans, then by perturbing it in the right way, it may be possible to extend normal youthfulness and lifespan.

So far, the only dauer-like trait that has not been uncoupled from longevity in *C. elegans* is stress resistance. Thus, it is possible that the ability to detoxify reactive oxygen species is what extends lifespan. This is plausible, because catalytic antioxidants are known to extend the lifespan of *C. elegans* (Melov et al., 2000) and because overexpression of superoxide dismutase (SOD) has been shown to extend the lifespan of *Drosophila* (Sun and Tower, 1999).

The *C. elegans* DAF-2 pathway acts non cell autonomously to regulate dauer formation and adult lifespan. The gene acts in neuroectoderm and, to a lesser extent, in internal organs, to produce one or more downstream signals or hormones which, in turn, regulate lifespan and dauer formation. DAF-2 also acts non cell autonomously to regulate adult fertility and intestinal fat metabolism. One downstream hormone may be a steroid ligand for DAF-12, a nuclear hormone receptor homolog that promotes dauer formation.

It is not known whether adult longevity, like dauer formation, can be regulated by environmental stimuli. This may be the case, since mutations and cell ablations affecting sensory neurons increase longevity, at least in part by modulating components of the insulin/IGF-1 pathway.

Insulin/IGF-1 Signaling in Drosophila

In *Drosophila*, an insulin/IGF-1 pathway regulates body size; animals carrying mutations in this pathway are small (reviewed in Weinkove and Leevers, 2000). This pathway also regulates lifespan. In *Drosophila*, as in vertebrates, an insulin-receptor substrate (IRS) protein couples receptor activation to PI3-kinase signaling. Null mutations in the *Drosophila* IRS homolog, *chico*, extend lifespan by ∼45% (Clancy et al., 2001). In addition, animals heterozygous for two different mutations in the insulin/IGF-1 receptor live 85% longer than normal (Tatar et al., 2001).

In addition to increased lifespan, the fly insulin/IGF-1 pathway mutants have another similarity to the *C. elegans* story. The animals appear to be in a state of reproductive diapause (Tatar et al., 2001). During the winter, flies do not reproduce, and egg development is arrested at previtellogenic stages. The *Drosophila* insulin/IGF-1 receptor mutants are sterile and their ovaries resemble the ovaries of wild-type flies in diapause.

Reproductive diapause is known to be regulated by juvenile hormone (JH), which is produced by a group of neurosecretory cells called the corpus allatum. Insulin/IGF-1 pathway mutants have low levels of JH. Treating these animals with methoprene, an analog of JH, can

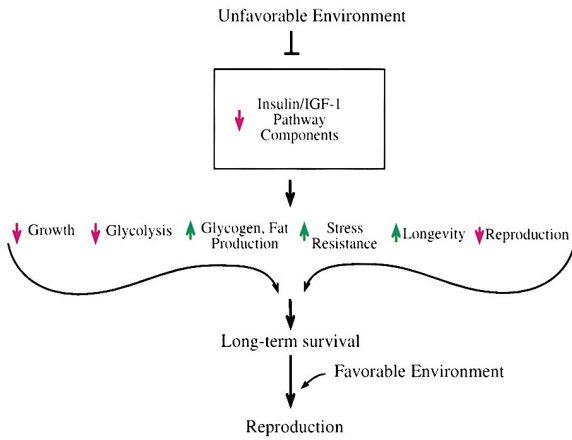*E-mail: ckenyon@biochem.ucsf.edu

Figure 1. Model for the Coordinate Control of Diapause-Linked Traits

These traits (green arrows) are upregulated during unfavorable conditions in response to decreased insulin/IGF-1 signaling. This, in turn, increases the chance that the organism will remain alive and still capable of bearing progeny when favorable conditions return. It is possible that increased stress resistance contributes to lifespan extension in some animals.

initiate vitellogenesis. Surprisingly, methoprene also restores wild-type lifespan to the long-lived insulin-receptor mutant, without changing the lifespan of wild-type flies (Tatar et al., 2001). This suggests that insulin/IGF-1 signaling stimulates JH production in the corpus allatum, which, in turn, regulates lifespan and reproductive diapause. The fly insulin/IGF-1 pathway is known to act cell autonomously to control body size (Bohni et al., 1999); however, this finding suggests that, as in worms, it acts non cell autonomously to regulate lifespan. JH may not be the only hormone that regulates lifespan in flies, because some insulin receptor mutants that appear to lack JH are not long-lived.

The long-lived flies have additional features reminis-

cent of diapause. They produce high levels of fat (Bohni et al., 1999, Tatar et al., 2001) and they express elevated levels of SOD (Clancy et al., 2001; Tatar et al., 2001). Again, it is informative to ask which of these traits can be uncoupled from lifespan extension. First, lifespan extension does not depend on the small size of the animals, because heterozygous *chico* null/+ animals are normal in size but long-lived (they live 36% longer than normal; Clancy et al., 2001). Reduced fertility is unlikely to cause longevity, because *chico(−)* females in which oocytes are removed by mutation live longer than *chico(+)* controls that also lack oocytes (Clancy et al., 2001). In addition, longevity is unlikely to be caused by reduced metabolism, because the metabolic rate of a long-lived insulin receptor mutant is normal (Tatar et al., 2001). In these flies, it is even possible to uncouple longevity from stress resistance. For example, *chico* mutants are long-lived but exhibit normal resistance to paraquat, a substance that generates reactive oxygen species (Clancy et al., 2001). This raises the possibility that longevity may not be caused by increased levels of oxygen detoxification after all.

While the parallels between the fly and worm insulin/IGF-1 systems are striking, the story is not so simple. With the exception of the *chico* null mutants, and one heteroallelic combination of insulin receptor mutations, none of the other mutations in this insulin/IGF-1 pathway extend lifespan (Clancy et al., 2001; Tatar et al., 2001). In fact, some shorten lifespan. Why might this be? First, it is possible that some components of the pathway simply do not function in lifespan regulation. In addition, some of the genes in the insulin/IGF-1 system are essential for viability. Thus, mutations that would otherwise extend lifespan may fail to do so because they also impair viability. The allele-specific pleiotropy of the *C. elegans* insulin signaling mutants makes this interpretation seem plausible, especially since many of the mutations in the worm insulin/IGF-1 receptor cause embryonic lethality. Perhaps in flies, only specific changes in
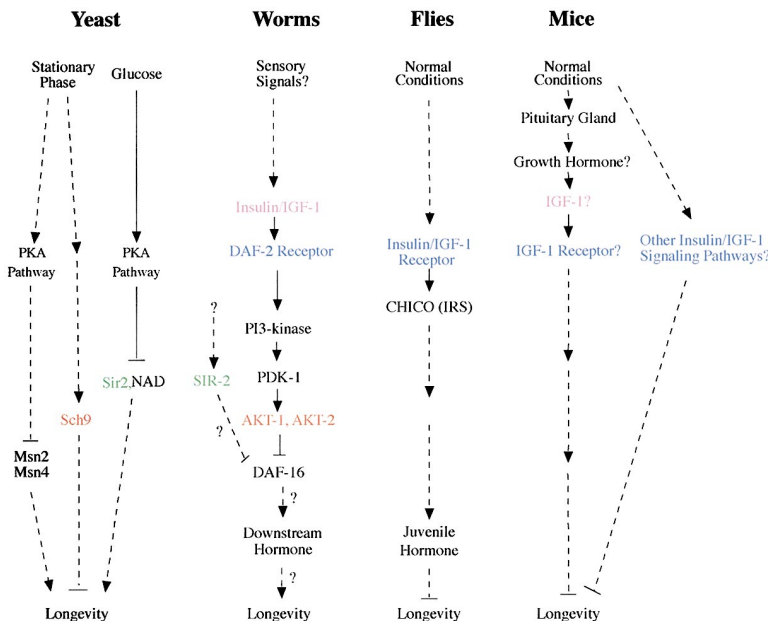

Figure 2. A Conserved System that Regulates Aging

Genes demonstrated (or strongly suspected) to regulate lifespan are shown. Question marks and dotted lines represent greater uncertainty. Homologs are color-coded.

signaling can extend lifespan without reducing viability. It might be interesting to vary the level of the different insulin/IGF-1 pathway components in a systematic way, and test for effects on lifespan.

Homologs of Insulin/IGF-1 Pathway Components in Yeast

Yeast, like metazoans, change their patterns of growth and physiology in response to their environment (reviewed in Thevelein and de Winde, 1999). When glucose levels are high, cells upregulate glycolysis, growth and proliferation. As glucose levels fall, the cells begin to divide more slowly and subsequently enter stationary phase. These cells synthesize food storage substances, upregulate stress resistance mechanisms, and either slow or cease reproduction. These alternative growth strategies are reminiscent of the alternative growth and diapause-like states of metazoans. In yeast, the response to glucose is mediated, at least in part, by two protein kinases, protein kinase A (PKA) and Sch9. PKA is activated when the level of cyclic AMP rises in response to glucose, and the Sch9 kinase is activated in an unknown fashion. The PKA pathway also regulates sporulation; mutants lacking PKA altogether sporulate even in the presence of food. Mutations in these genes not only shift cells into a state that more closely resembles metazoan diapause, they also increase lifespan. Reduction-of-function mutations in the PKA pathway extend the replicative lifespan of dividing cells; that is, they allow aging mother cells to undergo additional rounds of cell division before becoming senescent (Lin et al., 2000). Mutations affecting the PKA pathway also extend the postmitotic lifespan of cells in stationary phase, allowing nondividing cells to live longer than normal (Fabrizio et al., 2001). Mutations in *SCH9* extend the lifespan of stationary-phase cells quite dramatically, up to 3-fold (Fabrizio et al., 2001).

In order for mutations in the glucose response pathway to extend replicative lifespan, the cells require Sir2, an NAD-dependent histone deacetylase (Lin et al., 2000). Mutations predicted to reduce the level of NAD also block lifespan extension. Sir2 is likely to act downstream of PKA to regulate lifespan extension, because *SIR2* overexpression increases wild-type lifespan even in high glucose. It is not known whether Sir2 regulates the entire glucose response pathway, including food utilization pathways and the stress response, or whether it has a more specific role in regulating lifespan.

The parallels between the regulation of diapause-like states and lifespan in yeast and metazoans extend to the molecular level. First, the *C. elegans* homolog of *SIR2* has been shown to act in the *daf-2* insulin/IGF-1 pathway to extend worm lifespan (Tissenbaum and Guarente, 2001). Overexpression of *sir-2* potentiates dauer formation and extends lifespan in a *daf-16*-dependent fashion. In addition, *SCH9* encodes an Akt homolog (Kandel and Hays, 1999) suggesting that it, too, may have a common evolutionary ancestry with the insulin/IGF-1 pathway in *C. elegans.* (The *C. elegans* pathway contains two Akt homologs.)

In many metazoans, including vertebrates, caloric restriction alters food utilization pathways, increases stress resistance, extends lifespan, and postpones reproduction until food is restored. Thus, animals fed low levels of food have features in common with animals in diapause. Because the yeast PKA/Sch9 system regulates lifespan in response to glucose limitation, this system may mediate the effects of caloric restriction in yeast. Interestingly, it appears to be the cell's perception of glucose, rather than glucose consumption per se, that regulates lifespan. In the long-lived PKA pathway mutants, glucose is still present at high levels in the medium. Lowering PKA activity downregulates glycolytic enzymes and upregulates metabolic programs that are normally expressed in slow-growing cells or stationary phase cells. As a consequence, the rate of glucose catabolism would be predicted to fall. This metabolic shift might also be expected to raise the level of NAD (or the NAD/NADH ratio) in cells, thereby activating Sir2. Given its link to glucose sensation in yeast, it will be interesting to learn how *C. elegans sir-2* fits into the insulin/IGF-1 pathway in worms. Because the effects of *sir-2* overexpression are *daf-16* dependent, SIR-2 may act upstream of DAF-16 and the hormones or signals that DAF-16 controls. In vertebrates, the ratio of NAD/NADH appears to play a crucial role in regulating insulin release (Eto et al., 1999). Therefore, one possibility is that SIR-2 participates in a pathway that senses food or other environmental cues in *C. elegans* and regulates the release of a DAF-2 ligand.

How do the PKA and Sch9 pathways influence yeast lifespan? In dividing cells, *SIR2* overexpression and mutations in the PKA pathway decrease the production of rDNA circles (Guarente and Kenyon, 2000). rDNA circle formation has been shown to be sufficient to accelerate aging (although the mechanism is unknown); thus, it seems likely to be a cause, though perhaps not the only cause, of aging in these cells. In addition, it is possible that the perception of low glucose availability increases resistance to reactive oxygen species, which, in turn, increases lifespan. Mutations in the PKA pathway increase stress resistance by activating the transcription factors Msn2 and Msn4. If these activities are eliminated in PKA pathway mutants, resistance to oxidative stress is abolished; however, replicative lifespan is still increased (Lin et al., 1999). Thus, in dividing yeast cells, as in flies (which are largely postmitotic), stress resistance can be uncoupled from lifespan extension. Mutations in the PKA pathway also increase the lifespan and stress resistance of nondividing cells in stationary phase. Surprisingly, Msn2 and Msn4 are required for the lifespan extension of stationary-phase cells (Fabrizio et al., 2001). This is consistent with previous findings that overexpressing SOD can extend the lifespan of stationary-phase cells. Thus, different mechanisms may regulate lifespan extension in dividing and nondividing yeast cells. However, *sch9* complicates this interpretation, because no known mutations that decrease stress resistance in yeast can prevent *sch9* mutations from extending lifespan (relative to *SCH9(+)* controls; Fabrizio et al., 2001). Thus, it remains possible that the effect of sch9 on lifespan may be independent of the stress response.

The Big Picture

The similarities between these regulatory systems in worms, flies, and yeast are striking. First, each serves a similar biological function, allowing animals to postpone reproduction during unfavorable environmental conditions (Figure 1). Second, each regulates a similar set of

processes: oxidative stress resistance, food utilization pathways, reproduction, and lifespan. Third, the systems are composed, at least in part, of homologous genes and pathways (Figure 2).

Together these similarities suggest that this regulatory system arose early in evolution (see Kenyon, 1996; Tissenbaum and Guarente, 2001). Its selective value should be enormous. When food becomes limiting, an animal lacking this system would either die of starvation, or produce progeny that die of starvation. In contrast, with this food-sensing system in place, as food declines, the animal begins to build up fat and/or glycogen reserves, elaborates stress-resistance mechanisms, and delays or suspends reproduction until food is restored. It also activates pathways that extend lifespan, which increases the organism's chance of being alive and still youthful enough to reproduce if it takes a long time for conditions to improve. Each of the diapause-linked processes, such as production of food storage reserves, or the suspension of reproduction, would have selective value on its own. Enhanced stress resistance could protect the animal from heat or toxins that cause oxidative damage (Mahajan-Miklos et al., 1999). Because each has intrinsic value, there is no reason a priori for any one of these traits to cause lifespan extension. Thus, the fact that so many of these traits can be uncoupled from lifespan extension genetically in model organisms need not be surprising.

It is possible that this conserved system also operates in mammals (see Guarente and Kenyon, 2000; Clancy et al., 2001; Tatar et al., 2001). Mutant mice that lack the pituitary gland develop into long-lived dwarfs (Bartke, 2000). These mice have reduced levels of several hormones, including IGF-1 (Brown-Borg et al., 1996), which is produced in response to pituitary growth hormone. Interestingly, small dogs, which have low levels of IGF-1, live longer than large dogs. The finding that, in flies, the insulin/IGF-1 system regulates size and lifespan independently of one another suggests that these mutant mice and small dogs might be long-lived even if they were not small. Caloric restriction, too, may extend lifespan by impacting an insulin/IGF-1 pathway, since it reduces the level of circulating insulin and IGF-1. Rodents that are calorically restricted during development are small, but rodents that are calorically restricted during adulthood are normal in size but still live long. Thus, again, size and longevity can be uncoupled.

Insulin/IGF-1 pathways have increased greatly in complexity during evolution. Worms and flies have only one insulin/IGF-1 receptor, whereas vertebrates have at least three. Thus, it is possible that a lifespan regulatory function might be distributed among different branches of this endocrine system. In this regard, it is interesting that loss of the insulin receptor in the neuroepidermis decreases mouse fertility (Brüning et al., 2000). The same is true of worms (Apfeld and Kenyon, 1998), and possibly flies as well. It would be interesting to learn whether these mice, like the worms and flies, are long lived.

Aging proceeds at different rates in different animals. A mouse lives roughly two years; a bat, about 30–50; and a parrot, 90. How did these differences evolve? There must have been many mutations that increased lifespan during evolution, since all animals evolved from a common precursor that probably did not live very long. Longer lifespans may have evolved through changes in genes that regulate the rate of aging. For example, changes in insulin/IGF-1 pathway components could play a role in the evolution of lifespan, and this is suggested by the longevity of small dogs. This model is attractive because there is evidence that much of evolution occurs by changes in regulatory genes. In general, evolutionary biologists have not embraced the idea that aging is regulated, because there can be no selection for longevity once reproduction is complete and the progeny are able to live independently. However, the benefit of combining lifespan extension with other traits during diapause (particularly delayed reproduction) provides a strong justification for the evolution of a mechanism that regulates lifespan. If such a mechanism did not exist, the chance of an animal in diapause outliving harsh conditions before producing its progeny would be reduced. Because these insulin/IGF-1-like systems can link lifespan with reproduction, a mechanism for regulating lifespan would have selective value, and could arise during evolution.

Selected Reading

Apfeld, J., and Kenyon, C. (1998). Cell *95*, 199–210.

Bartke, A. (2000). In The Molecular Genetics of Aging, Vol. 29, S. Hekimi, ed. (Heidelberg: Springer-Verlag).

Bohni, R., Riesgo-Escovar, J., Oldham, S., Brogiolo, W., Stocker, H., Andruss, B.F., Beckingham, K., and Hafen, E. (1999). Cell *97*, 865–875.

Brown-Borg, H.M., Borg, K.E., Meliska, C.J., and Bartke, A. (1996). Nature *384*, 33–34.

Brüning, J.C., Gautam, D., Burks, D.J., Gillette, J., Schubert, M., Orban, P.C., Klein, R., Krone, W., Muller-Wieland, D., and Kahn, C.R. (2000). Science *289*, 122–2125.

Clancy, D., Gems, D., Harshman, L.G., Oldham, S., Stocker, H., Hafen, E., Leevers, S.J., and Partridge, L. (2001). Science *292*, 104–106.

Eto, K., Tsubamoto, Y., Terauchi, Y., Sugiyama, T., Kishimoto, T., Takahashi, N., Yamauchi, N., Kubota, N., Murayama, S., Aizawa, T., et al. (1999). Science *283*, 981–985.

Fabrizio, P., Pozza, F., Pletcher, S.D., Gendron, C.M., and Longo, V.D. (2001). Science. Published online Apr. 5, 2001; 10.1126/science.1059497.

Guarente, L., and Kenyon, C. (2000). Nature *408*, 255–262.

Kandel, E.S., and Hay, N. (1999). Exp. Cell Res. *253*, 210–229.

Kenyon, C. (1996). Cell *84*, 501–504.

Lin, S., Defossez, P., and Guarente, L. (2000). Science *289*, 2126–2128.

Mahajan-Miklos, S., Tan, M., Rahme, L.G., and Ausubel, F.M. (1999). Cell *96*, 47–57.

Melov, S., Ravenscroft, J., Malik, S., Gill, M.S., Walker, D.W., Clayton, P.E., Wallace, D.C., Malfroy, B., Doctrow, S.R., and Lithgow, G.J. (2000). Science *289*, 1567–1569.

Sun, J., and Tower, J. (1999). Mol. Cell. Biol. *19*, 216–228.

Tatar, M., Kopelman, A., Epstein, D., Tu, M., Yin, C., and Garofalo, R.S. (2001). Science *292*, 107–110.

Thevelein, J.M., and de Winde, J.H. (1999). Mol. Micro. *33*, 904–918.

Tissenbaum, H.A., and Guarente, L. (2001). Nature *410*, 227–230.

Weinkove, D., and Leevers, S.J. (2000). Curr. Opin. Genet. Dev. *10*, 75–80.