Extension of Life-Span in *Caenorhabditis elegans* by a Diet Lacking Coenzyme Q

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The isoprenylated benzoquinone coenzyme Q is a redox-active lipid essential for electron transport in aerobic respiration. Here, we show that withdrawal of coenzyme Q (Q) from the diet of wild-type nematodes extends adult life-span by ~60%. The longevity of *clk-1*, *daf-2*, *daf-12*, and *daf-16* mutants is also extended by a Q-less diet. These results establish the importance of Q in life-span determination. The findings suggest that Q and the *daf-2* pathway intersect at the mitochondria and imply that a concerted production coupled with enhanced scavenging of reactive oxygen species contributes to the substantial life-span extension.

Coenzyme Q (Q) functions as a carrier of electrons and protons in respiratory complexes I, II, and III. Electron transport by complexes I and III establishes a proton gradient across the inner mitochondrial membranes, which is coupled to synthesis of adenosine triphosphate (ATP) by the F_0/F_1 ATPase (complex V). Q also functions in cells as a lipid-soluble antioxidant, as a redox-active component of plasma-membrane electron transport, in uridine synthesis, and as a cofactor for the proton-pumping activity of mitochondrial uncoupling proteins (*1*, *2*). *Caenorhabditis elegans clk-1* mutants lack the endogenously synthesized isoform Q_9 (where 9 designates the number of isoprene units in the polyisoprene tail) and instead rely on the *Escherichia coli* isoform Q_8, which is supplied by their standard diet for growth (*3*). The *clk-1* gene encodes a di-iron carboxylate enzyme responsible for the final hydroxylase step in the synthesis of Q (*4*). The *clk-1* mutant animals accumulate the Q-biosynthetic intermediate demethoxy-Q_9 (DMQ_9) (*5*). These findings suggest that the increased life-span and slowed rates of development reported for *clk-1* mutants result from a decreased level of Q.

To test whether a decrease in dietary Q alters life-span, we fed a diet lacking Q to wild-type worms during the adult phase. The growth and adulthood phases are separated in *C. elegans* by a molt from the fourth larval stage (L4) to adult worm. Therefore, to avoid altering development from standard conditions, we switched larvae from the standard diet of OP50, a Q_8-replete *E. coli*, to a diet of GD1, a Q-less *E. coli*, at the L4 stage. Wild-type adults fed the Q-less diet throughout adulthood had a median life-span 59% longer than those fed standard Q-replete *E. coli* (Fig. 1A and Table 1). A Q-less diet also extended life-span for *fer-15(b26ts)* nematodes, which carry a temperature-sensitive mutation that causes sterility. When either wild-type or *fer-15(b26ts)* nematodes were fed the GD1: pAHG *E. coli* strain (which is rescued for the *ubiG* gene and thereby rendered Q_8-replete), the life-span was similar to those of animals fed OP50. These results indicate that the short life-span does not result from the genetic background of the OP50 *E. coli* strain and is unrelated to the presence or absence of kanamycin in the growth medium. Rather, the short life-span of wild-type and *fer-15* mutant animals appears to depend on a dietary source of Q.

The GD1 *E. coli ubiG* mutation causes accumulation of 2-octaprenyl phenol, an intermediate specific to the Q-biosynthetic pathway in prokaryotes (*6*). To test whether the increased longevity depended on this intermediate rather than on the absence of Q, we used a Q-less *E. coli ubiE* mutant strain that accumulates 2-polyprenyl-6-methoxy-1, 4-benzoquinol as food for wild-type worms. Because this *ubiE* mutation was present in JC7623, a different *E. coli* genetic background than that of GD1, we included JCΔG, a strain harboring the *ubiG* mutation in the JC7623 genetic background, as a control (*6*). An increase in nematode life-span was observed

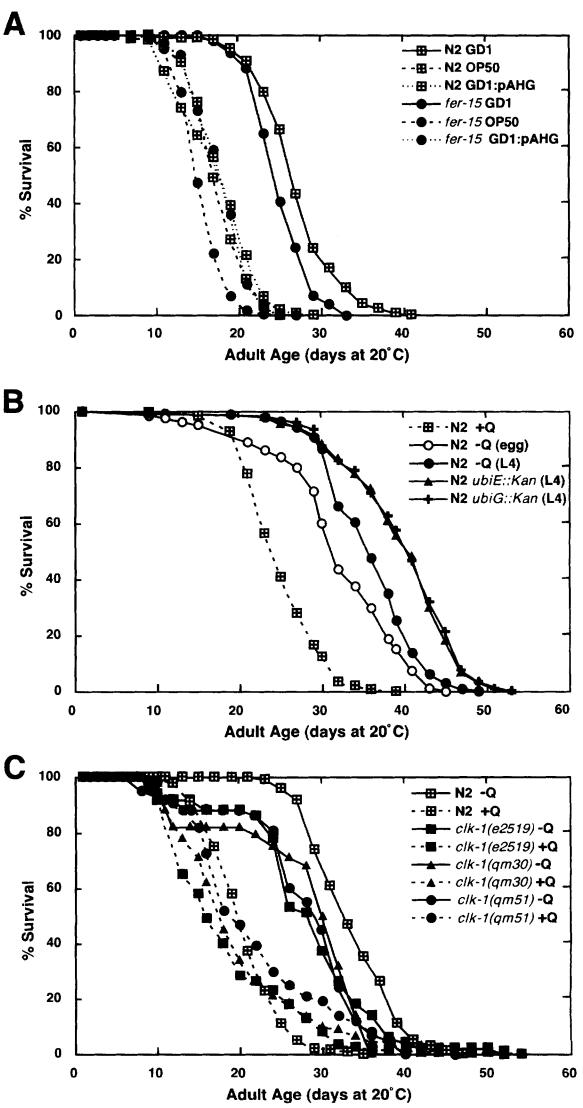

Fig. 1. Adult *C. elegans* life-span is extended by a diet of Q-less *E. coli*. (**A**) Survival curves of wild-type N2 (cross-hatched squares) and *fer-15(b26ts)* (filled circles) adults fed either Q-less or Q-replete *E. coli* throughout their adult life. The diets were standard Q_8-replete *E. coli* OP50 (dashed lines); GD1, a Q-less strain of *E. coli* (solid lines); or rescued GD1:pAHG, a Q_8-replete strain of *E. coli* (dotted lines). All experiments with non-OP50 *E. coli* contained kanamycin in the plate media to kill Q-replete OP50 that accompanied the worms upon transfer to the Q-less food. (**B**) Adult life-span survival curves of N2 fed a variety of Q-less *E. coli* diets. Diets from hatching and throughout adulthood were standard Q_8-replete *E. coli* OP50 (cross-hatched squares) or the Q-less GD1 *E. coli* strain, harboring a mutation in *ubiG* (open circles). L4 larvae were transferred to a Q-less diet of GD1 (filled circles); JC7623Δ4-1, *ubiE*::Kan (triangles); or JCΔG, *ubiG*::Kan (crosses). (**C**) Survival curves of wild-type N2 (cross-hatched squares), *clk-1(e2519)* (filled squares), *clk-1(qm30)* (triangles), and *clk-1(qm51)* (circles) adults fed either Q-less or Q-replete bacteria throughout their adult life. The lines are as described in (A). The *clk-1* mutations have been sequenced (*23*), and *e2519* is predicted to affect a carboxylate ligand (E148K) (*4*) whereas the other alleles result in truncated polypeptides.

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for each of the mutant *E. coli* strains, suggesting that the observed life-span extension was independent of the type of Q-biosynthetic intermediate that accumulated (Fig. 1B and Table 1). These results imply that a lack of Q_8 extends life-span.

Wild-type animals were fed the Q-less diet from egg to old age so that we could investigate the effect of the absence of Q during development on adult aging. A small fraction of this adult population died earlier than did animals raised on the Q-replete diet (Fig. 1B). Although most of the remaining nematodes raised and maintained on the Q-less diet survived longer than those raised and maintained on the Q-replete diet, they had decreased median survival when compared with nematodes switched as L4 larvae to the Q-less food. Of the nematodes raised on Q-less food, 45 out of 165 had reproductive defects, whereas of the nematodes raised on Q-replete food and switched to the Q-less diet as L4 larvae, only 21 out of 181 had reproductive defects (*7*). These defects are presumed to be developmental, because that is the period during which the reproductive structures are formed. This suggests that dietary Q is beneficial during development and engenders formation of robust adults. These results suggest that, although development proceeds more reliably with Q in the diet, adult consumption of Q_8 shortens nematode life-span.

Table 1. Life-span analysis of *C. elegans* fed different *E. coli* diets. Independent experiments are in groups beginning with N2.

Food	Median (days) ± SE	Maximum (days)	Number of deaths†	% of N2 on OP50‡	% of same strain on OP50‡
		N2, 20°C			
OP50	17 ± 0.36	25	133	–	–
GD1	27 ± 0.41	41	127	159**	159**
Rescued GD1	19 ± 0.48	29	137	112	112
		fer-15(b26), 20°C			
OP50	15 ± 0.27	27	162	88**	–
GD1	25 ± 0.48	33	100	147**	167**
Rescued GD1	19 ± 0.33	25	152	112	127**
		N2, 20°C			
OP50	25 ± 0.57	39	147	–	–
GD1 (egg)	32 ± 0.99	45	80	128**	128
GD1 (L4)	36 ± 0.66	49	142	144**	144
ubiE⁻	41 ± 0.63	53	151	164**	164
ubiG⁻	41 ± 0.71	53	122	164**	164
		daf-12(m20), 20°C			
OP50	19 ± 0.80	36	129	76**	–
GD1	29 ± 0.42	43	121	116**	153**
		daf-16(m26), 20°C			
OP50	21 ± 0.27	25	154	84**	–
GD1	29 ± 0.35	36	171	116**	138**
		N2, 20°C			
OP50	21 ± 0.46	35	136	–	–
GD1	33 ± 0.91	47	74	157**	157**
		clk-1(e2519), 20°C			
OP50	18 ± 0.88	54	124	86	–
GD1	30 ± 1.06	52	51	143**	167**
		clk-1(qm30), 20°C			
OP50	18 ± 0.67	48	110	86	–
GD1	30 ± 1.06	36	28	143**	167**
		clk-1(qm51), 20°C			
OP50	20 ± 1.45	46	104	95*	–
GD1	30 ± 0.99	40	42	143**	150*
		N2, 25°C			
OP50	16 ± 0.36	28	168	–	–
GD1	20 ± 0.45	26	95	125**	125**
		daf-2(m41), 25°C			
OP50	28 ± 1.02	44	126	175**	–
GD1	34 ± 0.75	50	96	213**	121**
		daf-2(e1370), 25°C			
OP50	30 ± 1.09	46	76	188**	–
GD1	38 ± 1.56	54	61	238**	127**
		clk-1(qm30), 25°C			
OP50	14 ± 0.37	22	121	88**	–
GD1	20 ± 0.67	28	89	125**	143**
		daf-12(m20), 25°C			
OP50	12 ± 0.32	18	128	75**	–
GD1	14 ± 0.26	22	129	88**	117**
		daf-2(e1370); daf-12(m20), 25°C			
OP50	36 ± 0.86	56	141	225**	–
GD1	34 ± 1.21	54	119	213**	94
		daf-2(e1370) clk-1(qm30), 25°C			
OP50	46 ± 1.12	68	151	288**	–
GD1	52 ± 1.53	84	132	325**	113**

†Animals that crawled away, had internally hatched larvae, or had eviscerated gonads were excluded. ‡Log rank test for survival analysis *P* values: *$P = 0.01$, **$P \leq 0.0001$.

The short life-span of wild-type animals fed OP50 is considered normal for *C. elegans*. Animals with mutations in previously described life-span–determination genes (*clk-1* and *daf*) were tested for a long-lived (Age) phenotype when fed Q-less food. *clk-1(qm30, qm51,* and *e2519)* mutant animals were transferred to the Q-less diet as L4 larvae, and adult life-span was determined. Mutant strains fed the Q-less diet showed increases in median life-span relative to the same genotype fed the Q-replete diet (Fig. 1C and Table 1). However, insufficient Q appears to be detrimental because, regardless of diet, nearly all the *clk-1* animals, which do not synthesize Q, died at younger ages than did the wild-type animals on a Q-less diet. The *clk-1*–mutant animals fed the Q-replete diet showed increased survival when compared with wild-type animals only at the oldest ages (Fig. 1C) (*7*). The median life-span for the *clk-1*–mutant population fed the Q-replete diet was decreased relative to that of the wild type. This result differs from a reported 7 to 20% increase in mean life-span for *clk-1* (*8*, *9*). These published analyses describe animals that started from eggs and include the increased developmental time of the *clk-1* mutants in the number of days lived, whereas our experiments start on the first day of adulthood. A decrease in Q levels, whether due to a Q-less diet or a mutation in the *clk-1* gene, results in longevity.

In *C. elegans*, the *daf* genes regulate both development and life-span (*10*). This longevity pathway includes the *daf-2* and *age-1* genes that increase life-span when mutated. Participation of these gene products in an insulin-like signaling pathway is inferred from their molecular identity with mammalian homologs (*11*, *12*). Traditionally, suppression analysis is used to determine the gene products required for a particular phenotype. For example, the longevity phenotype of *daf-2(e1370)* is considered to be suppressed by *daf-16* because the double-mutant *daf-16(m26); daf-2(e1370)* is short-lived. Hence, *daf-2* mutant longevity requires wild-type DAF-16 activity (*10*, *13*). By similar criteria, the life-span extension by laser ablation of germ line precursor cells requires wild-type

DAF-16 and DAF-12 activities (*14*). We performed suppression tests for the Age phenotype due to withdrawal of dietary Q. As previously observed, when fed a Q-replete diet, the *daf-16(m26)* and *daf-12(m20)* adults have shorter life-spans than wild-type animals (Fig. 2A and Table 1). The life-spans of the *daf-16* and *daf-12* mutants fed a Q-less diet were longer than those of wild-type animals fed a Q-replete diet. Neither the *daf-12(m20)* nor the *daf-16(m26)* mutation suppressed the life-span extension generated by the Q-less diet. Thus, the *daf-12* and *daf-16* gene products are not required for the longevity effect mediated by the diet of Q-less bacteria.

We examined the effect of a Q-less diet on the life-span of long-lived *daf-2* mutants. The restrictive temperature, 25°C, was used to obtain the largest increase in life-span relative to that obtained with the wild type (*10*). Under these conditions, both *daf-2(e1370)* and *daf-2(m41)* adults displayed longer median and maximum life-spans when fed a Q-less as compared with a Q-replete diet (Fig. 2B and Table 1). The *C. elegans* strains with the longest life-spans are double mutants of *daf-2(e1370)* with mutations in either the *daf-12* or *clk-1* gene (*8*, *10*). The *daf-2(e1370)* and *clk-1* longevities are additive, whereas *daf-2(e1370)* and *daf-12* are synergistic (*8*, *10*). The long-lived double mutants were tested along with the single-mutant controls on Q-less and Q-replete diets. There was no increase for *daf-2(e1370); daf-12(m20)* fed the Q-less food (Fig. 2C and Table 1). There was a small increase for *daf-12(m20)*, and perhaps it is this phenotype that we observed in the *daf-2(e1370); daf-12(m20)* fed a Q-less diet for which a 2-day increase of an already long life-span was not significant. The increased median life-span of *daf-2(e1370) clk-1(qm30)* animals was further extended when they were fed a diet lacking Q. Thus, the longevity mechanisms are additive. There are two possible interpretations with regard to mechanism. Either DAF-2 signaling and decreased Q constitute separate parallel pathways, both needed for a later step, or they act serially in a single pathway, each with a partial effect.

For wild-type nematodes, the percent life-span extension resulting from the Q-less diet relative to the Q-replete diet was smaller at 25°C than it was at 20°C (Table 1). The N2 genotype had increased oxygen consumption at 25°C (*15*), and the number of mitochondria in muscle cells was nearly doubled in animals raised at 25°C compared with animals raised at 15°C (*16*). Thus, increased respiration and mitochondrial biogenesis at 25°C may require higher levels of Q. These observations suggest that the site of action of the Q-less food on longevity is the mitochondria.

Overall, dietary Q is not necessary for survival, but the animals' dietary needs differed, depending on their stage of development. Dietary Q is essential during larval stages for the *clk-1*–mutant animals to develop into fertile adults (*3*), but in its absence the arrested larvae survived for weeks with only the maternal contribution of Q. In adulthood, the postmitotic animals of all genotypes tested survived with or without exogenous Q. A Q-less diet may trigger an adaptive response with physiological and biochemical changes that benefit longevity. Our data suggest a model in which dietary Q and insulin-like signaling are parallel pathways that converge to influence the mitochondrial production of reactive oxygen species (ROS) (Fig. 3). Both dietary Q and Q produced by de novo biosynthesis would be expected to function within the mitochondria and have a direct effect on respiratory-chain efficiency (Fig. 3, A to D). Q is also an essential cofactor for the H^+ pumping of each of the UCP1, UCP2, and UCP3 uncoupling proteins (*2*), which may play a role in controlling ROS production by mitochondria (*17*). In our model, decreased Q levels result in reduced generation of oxidative damage, which is predicted to increase life-span. The changes downstream of the *daf-2* insulin-like receptor are predicted to control respiration by a variety of indirect mechanisms (Fig. 3D). Intermediary metabolism is altered in dauer larvae and *daf-2* mutant adults (*18*–*20*) such that limitation of oxidizable substrates would control respiration rates. DAF-2 signaling would regulate transcription of genes involved in respiratory or fermentative metabolism. Finally, the *daf-2/age-1* pathway mutant longevity is associated with increased ROS scavenging ability in the long-lived mutants (*20*–*22*). Although mitochondria are a common focal point for the Q-less food and *daf-2* mutant longevity mechanism proposed, there are metabolic alterations unique to each condition. For example, the *clk-1* mutants have higher levels of RQ_9 (*3*), and the increase in RQ_9 would be expected to shift metabolism toward anaerobic fermentation, which would be expected to decrease ROS. Thus, the combination of reduced generation and increased scavenging mechanisms are predicted to result in a substantial decrease in the total cellular ROS and thereby allow for an extended life-span.

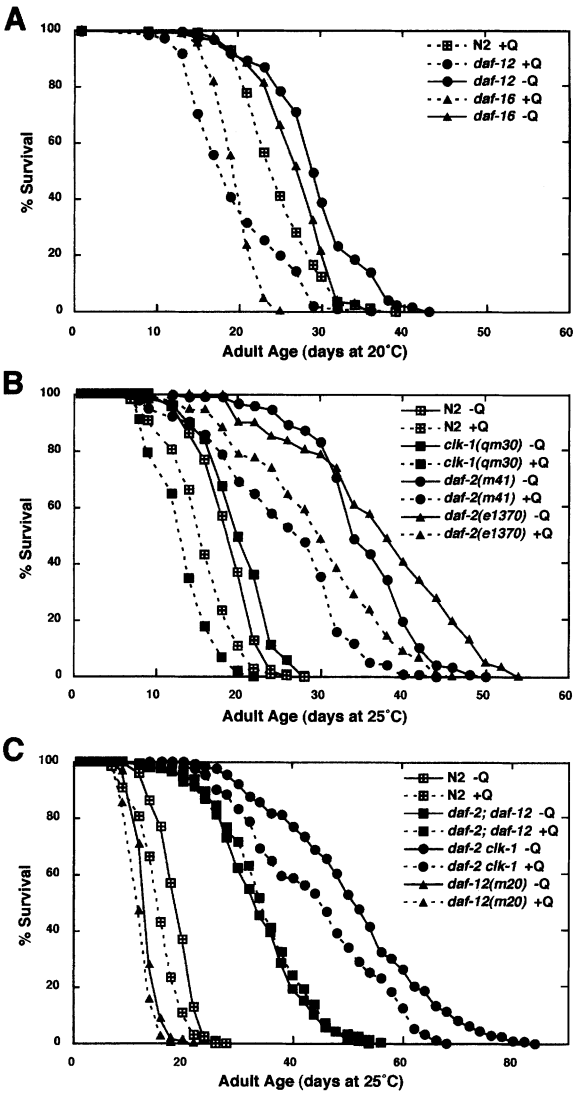

Fig. 2. Life-span of *daf* mutant animals is extended by withdrawal of dietary Q. (**A**) Survival curves of N2 (cross-hatched squares), *daf-12(m20)* (circles), and *daf-16(m26)* (triangles) adults fed either Q-less or Q-replete *E. coli* throughout their adult life. The diets were standard Q-replete *E. coli* OP50 (dashed lines) or GD1, a Q-less strain of *E. coli* (solid lines). (**B**) Survival curves of N2 (cross-hatched squares), *clk-1(qm30)* (filled squares), *daf-2(m41)*, a mutation in the ligand-binding domain (*24*) (circles), and *daf-2 (e1370)*, a mutation in the kinase domain (*11*) (triangles) adults. The lines are as described in (A). Animals were raised at 15°C and fed OP50 until reaching L4. L4 larvae were transferred to the restrictive temperature (25°C) and were fed either Q-less or Q-replete *E. coli* throughout their adult life. (**C**) Survival curves of N2 (cross-hatched squares), *daf-2 (e1370); daf-12(m20)* (filled squares), *daf-2(e1370) clk-1(qm30)* (circles), and *daf-12(m20)* (triangles) adults. The lines are as described in (A). The animals were handled as described in (B).

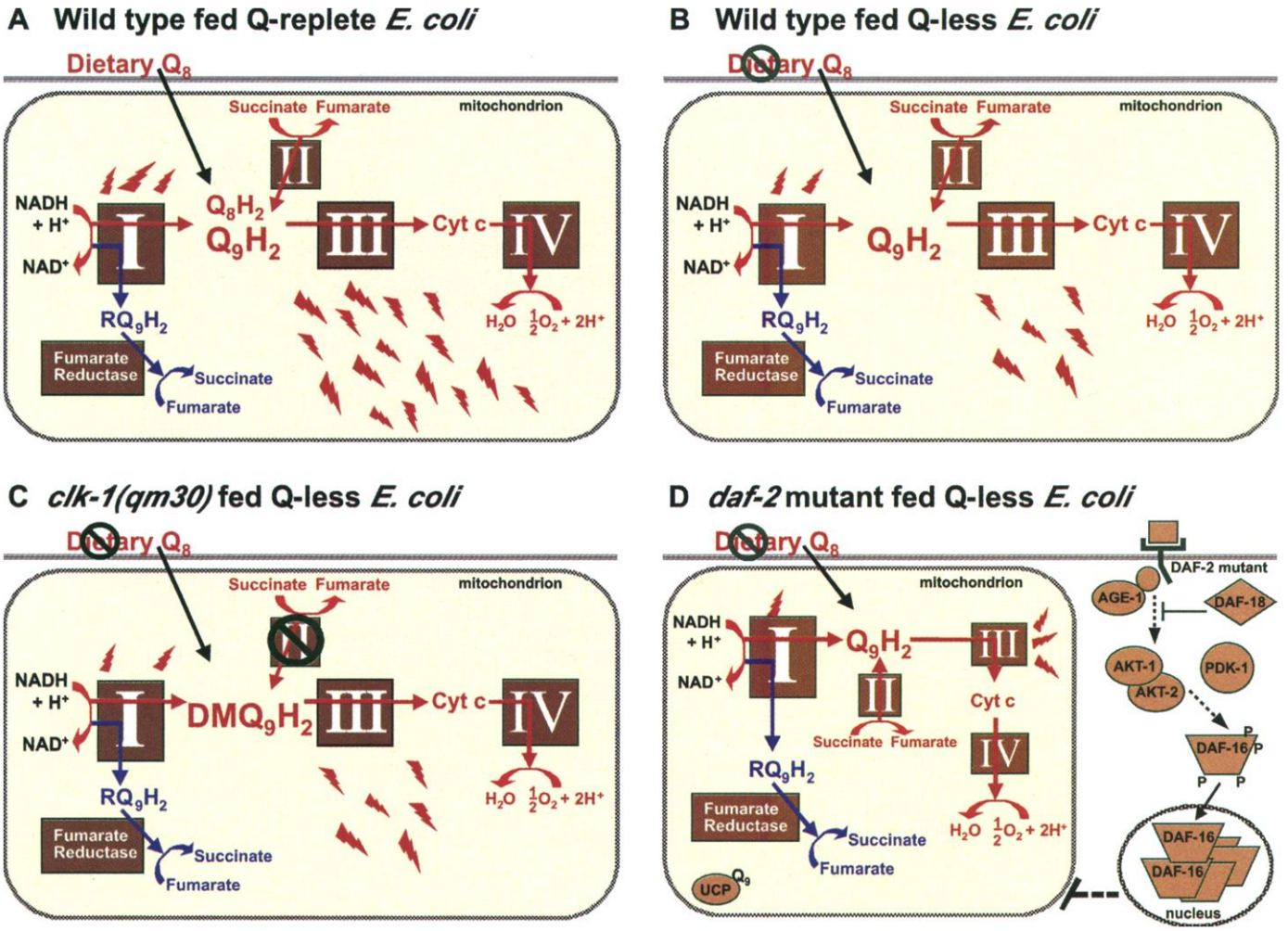

Fig. 3. A model relating the withdrawal of dietary Q_8 to life-span extension in three *C. elegans* genotypes. Q within the mitochondria is derived from either de novo synthesis (Q_9) or from the diet (Q_8). The nematode respiratory chain (complexes I, II, III, and IV) is shown. Red arrows depict aerobic respiration (electron transport to oxygen). The associated production of ROS is designated by red bolts, and the amount of ROS is represented by the number and size of the bolts. Blue arrows depict nematode anaerobic respiration with RQ_9, an amino quinone that functions in complex I; instead of carrying electrons to complex III, RQ_9H_2 is oxidized by fumarate reductase, providing a bypass of O_2 as a terminal electron acceptor. (**A**) Under standard growth conditions, wild-type animals contain Q_9, RQ_9, and Q_8 (*3*). (**B**) N2 animals transferred to a Q-less diet produce Q_9 and RQ_9. When the animals are transferred to the Q-less diet, withdrawal of Q_8 from the diet is proposed to lead to decreased ROS production. (**C**) *clk-1* mutants transferred to the Q-less diet as L4 larvae and maintained on this diet. The quinones present probably would consist only of RQ_9 and DMQ_9. Based on functional studies of DMQ_8 in *E. coli* (*25*), it seems likely that DMQ_9 may retain partial function in nematode complex I but is inactive in complex II. Withdrawal of Q_8 from the diet is proposed to lead to decreased ROS production. (**D**) In addition to the mitochondrion, the insulin-like signal transduction pathway is depicted on the right side of the panel (*26*). The dashed lines signify that the *daf-2* mutations cause reduced signaling, rather than the absence of signaling. The T-bar represents inhibition of mitochondrial respiratory activity after an as yet unknown number of steps. In this situation, the mutation is proposed to lead to increased ROS scavenging and slightly decreased ROS production relative to that of the wild type [in (A)]. Withdrawal of Q_8 from the diet is proposed to lead to decreased ROS production, and this is additive to the increased ROS scavenging and slightly decreased ROS production that result from mutation of the *daf-2* gene.

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