specific sites in promoters of autophagy-related genes and that fasting indeed increases such FoxO3 binding. Conversely, the authors provide convincing evidence that knockdown of FoxO3 prevents starvation-induced autophagy in isolated fibers and in muscle in vivo.

Mammucari et al. (2007) further investigate the mechanisms of this regulation and discover that *Bnip3*, whose transcription is controlled by FoxO3, plays a major role in mediating the effects of FoxO3 and starvation on autophagy. Bnip3 was previously found to induce autophagy in cardiac myocytes, where strikingly, this regulation depends on Bnip3 localization to the mitochondrial outer membrane (Hamacher-Brady et al., 2007). Further research is therefore required to sort out how much autophagy is induced as a consequence of the mitochondrial functions of Bnip3. Nonetheless, the relevance of autophagy to muscle physiology is undoubtedly emphasized by the observation that knockdown of Atg8/LC3 partly reduces muscle atrophy.

Together, these findings demonstrate that FoxO3 directly and coordinately activates the lysosomal and proteasomal pathways in muscle wasting. Critical proteins targeted for breakdown by either system remain to be identified. Whether FoxO3 may control alternative proteolytic routes (e.g., calpains and caspases) that also seem to be important in muscle wasting remains to be determined.

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Mitochondria and Aging: Dilution Is the Solution

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DOI 10.1016/j.cmet.2007.11.008

Metabolic component depletion in model systems results in life-span extension, which has been difficult to reconcile with human metabolic pathologies. Recently, Rea et al. (2007) have shown that mitochondrial electron transport chain RNAi phenotypes in the worm *C. elegans* are dose dependent, providing an alternative view of mitochondrial function in longevity and metabolic diseases.

Mitochondria not only are the "powerhouses of the cell" but also contribute to numerous cellular functions, including generation of free radicals, apoptosis, calcium signaling, and at least in the worm *C. elegans*, aging. Abnormalities in these dynamic organelles are also associated with various human metabolic diseases. The discrepancies between human metabolic disorders and the phenotypes displayed by *C. elegans* with knocked down or mutated electron transport chain (ETC) components have been a source of confusion for quite some time. While the human metabolic conditions result in detrimental phenotypes such as seizures, ataxia, exercise intolerance, cardiomyopathies, and shortened life span, *C. elegans* ETC mutants show a surprising variety of phenotypes ranging from larval-arrested (*atp-2(ua2)*, *nuo-1(ua1)*) to short-lived (*mev-1(kn1)*, *gas-1(fc21)*) to long-lived (*clk-1(qm30)*, *isp-1(qm150)*) (Rea, 2005). Worms treated with RNAi against numerous different nuclear-encoded mitochondrial ETC and ATP synthase genes often exhibit lengthened life span (Dillin et al., 2002; Lee et al., 2003; Curran and Ruvkun, 2007; Chen et al., 2007). Rea et al. (2007) attempt to resolve the disparity between

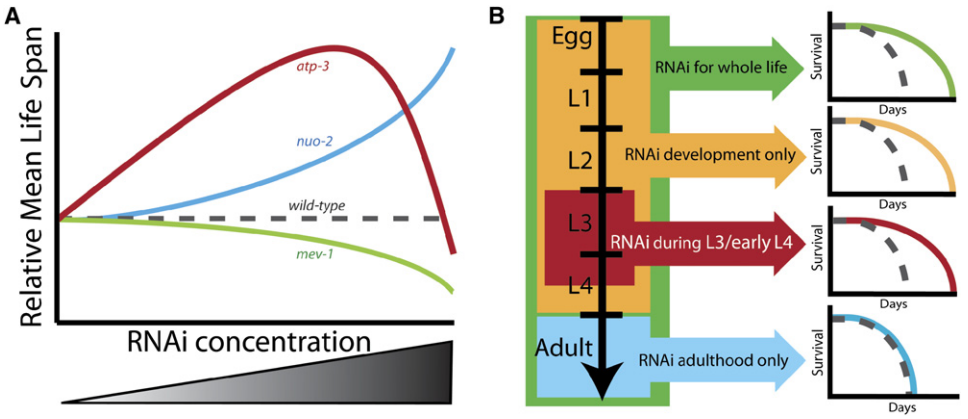

Figure 1. Dilution and Timing of Mitochondrial Electron Transport Chain RNAi Determines Longevity

(A) Different RNAi concentrations result in different mean life-span curves. Relative mean life-span curves resulting from electron transport chain (ETC) component knockdown via RNAi at increasing concentrations of dsRNA-expressing bacteria are depicted. *atp-3* RNAi, shown in red, has a peak increase in life span at a 1:10 dilution, while undiluted concentrations prove to be detrimental to life span. *nuo-2* RNAi (blue) exhibits an increasing life-span response with increasing concentrations of RNAi bacteria. *mev-1* RNAi (green), which targets an ETC gene that has not been shown to cause increased life span, does not exhibit an increase in life span at any concentration of RNAi.

(B) Timing requirements for ETC RNAi. *C. elegans* life span from egg through four larval stages to adulthood is shown at the left. Colored areas represent different times/stages that RNAi toward ETC components is administered. Feeding RNAi throughout the whole life (green area) results in an increased life span (green line). RNAi knockdown during development only (yellow area) increases life span (yellow line). RNAi for the L3/L4 window of development (red area) also increases life span, whereas RNAi during adulthood only (blue area) has no effect on life span.

increased life span in worms due to ETC knockdown and similar defects in humans that result in disease. The authors explored the hypothesis that increased longevity due to deficiencies within the ETC follows a bell-shaped curve (Figure 1A), a proposed "mitochondrial threshold effect." Using dilution-based dsRNA for the ETC gene *atp-3*, the oligomycin sensitivity-conferring protein/delta subunit of the ATP synthase, a window of increased life span was observed, suggesting that a mitochondrial threshold effect may be largely responsible for the contrasting phenotypes.

The mitochondrial threshold effect theory suggests that mitochondria are able to function under compromised conditions (for example, low levels of mutated mtDNA or heteroplasmy) up to a certain point. Beyond this threshold, the cells are no longer able to cope and suffer a loss of mitochondrial function and cell viability (Wallace, 1986). This theory attempts to explain the elusive relationship between metabolic genotype and the resulting phenotype.

The recent work by Rea and colleagues (2007) employs a 12-point dilution series of bacteria expressing dsRNA toward the gene of interest. For *atp-3*, they found that the maximal life-span extension of 70% was observed at a 1:10 dilution. At higher concentrations of *atp-3* dsRNA, the worms were very small and life span was shortened (depicted in Figure 1A). Based on this evidence, the authors propose that under certain circumstances of partial knockdown due to RNAi dilution, maximum life span can be achieved.

Interestingly, however, the other mitochondrial ETC genes associated with longevity that were tested, *cco-1*, *isp-1*, and *nuo-2*, also showed a graded increase in life span, but none exhibited a life span shorter than wild-type, even when fed undiluted RNAi. The authors also took advantage of RNAi-hypersensitive mutant worm strains; however, treatment of these strains with RNAi against *cco-1*, *isp-1*, and *nuo-2* did not result in the bell-shaped life-span curve observed for *atp-3* RNAi, nor did it result in a shift in sensitivity. Therefore, there could be a detrimental side effect (shortened life span) to severe knockdown of the ATP synthase that is not shared with severe knockdown of complex I, III, and IV components.

Metabolic theories of aging often cite the fact that mitochondria produce most of the cell's damaging free radicals as an explanation for the observed effects. The "free radical theory of aging" could explain the ETC RNAi life-span results or phenotypes in terms of a proposed oxidative stress response or reduced free radical production. However, Rea et al. (2007) and others (Lee et al., 2003) have yet to find a connection between oxidative stress and the longevity phenotype. In the current study, they find no significant correlation between the amount of protein oxidative damage and longevity of worms fed *atp-3* RNAi. Moreover, they did not observe decreased or left-shifted life span in *skn-1* mutant worms, which exhibit increased oxidized protein levels, because *skn-1* is a key transcription factor responsible for regulating oxidative stress defense (An and Blackwell, 2003). Therefore, the growing body of results from different groups suggests that it is not that the stochastic accumulation of free radicals throughout the life span of an animal accelerates the aging process. Alternatively, a different metabolic byproduct of mitochondrial metabolic activity, distinct from free radicals, may be at the source of aging.

An underlying feature of the "rate of living" theory of aging suggests that the amount of the increase in life

span due to reduced metabolic output is correlated to the amount of time that metabolism is actually reduced. This aspect of the metabolic theory was questioned when it was discovered that mitochondrial function only needs to be reduced during the larval stages of the developing worm for life-span extension to occur (Dillin et al., 2002). From this work, it was hypothesized that during larval development, a regulatory signal is established and somehow maintained for the remainder of the animal's life, even if the missing mitochondrial components are reinstated (Figure 1B). Expanding upon this work, Rea et al. (2007) further define the timing of this execution point to a specific period during the worm's larval development, the L3/L4 period of the life cycle. Much like in previous studies, RNAi of mitochondrial components during adulthood, no matter what the dilution, had no effect on life span.

It will be of critical importance to determine precisely what is monitored and established during the L3/L4 larval stage as the signal for mitochondrial perturbation that results in increased longevity. Tsang and Lemire (2002) previously proposed an energy-sensing checkpoint that monitors mitochondrial status as an explanation for the L3/L4 arrest associated with mitochondrial mutations or other mitochondrial perturbations. It is during this time period that energy demands are high due to the proliferating germline and that, indeed, mitochondrial numbers in the worm expand the most (Tsang et al., 2001). If energetic requirements cannot be met, larval arrest results. The L3/L4 arrest leads Rea and colleagues (2007) to propose a cell-cycle role in the mitochondrial longevity phenotype. This is an interesting model given that p53 has recently been shown to regulate mitochondrial activity (Matoba et al., 2006). However, this cell-cycle-based model would have to take into account specific cell types as being responsible for the mitochondria-related longevity because by the L3/L4 transition, almost all of the 959 cells of the animal are postmitotic. Therefore, the cell-cycle regulation of ETC-mediated longevity would likely have to be cell type specific. In the future, it will be important to understand the energetic requirements during this transition that might serve as the signal to reduce the aging process and the cells and tissue types in which reduced mitochondrial function must act to promote longevity.

In conclusion, the role of mitochondrial function and longevity is turning out to be much more complex than originally postulated. The work of Rea et al. (2007) raises important points about the relationship between mitochondrial component knockdown and human pathologies, as well as wide-reaching issues for RNAi. It illuminates the necessity of considering the level of RNAi administered and the analysis of the phenotypic threshold. This work provides the basis for screening or reevaluating genes that cause larval arrest in a way that recreates something of a phenotypically "weak allele." Apparently, not only the level of mitochondrial gene reduction must be finely tuned, but also the timing of reduction as well, to generate specific life-span phenotypes.

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