The redshift of PAx with respect to \( \lambda_{13} \).


27. References and Notes

1. O. Isler, Carotenoids (Birkhauser-Verlag, Basel, 1971).


24. With respect to previously reported experiments, this would result in large differences in the electronic designation of Sx and its involvement in the molecular mechanisms of carotenoid-to-chlorophyll singlet-singlet energy transfer remain to be determined.

25. Supporting Online Material

www.sciencemag.org/cgi/content/full/298/5602/2395/DC1

Materials and Methods

Fig. S1

References and Notes

4 June 2002; accepted 7 November 2002

Rates of Behavior and Aging Specified by Mitochondrial Function During Development

Andrew Dillin,1* Ao-Lin Hsu,1 Nuno Arantes-Oliveira,†† Joshua Lehrer-Graiwer,1 Honor Hsin,1‡ Andrew G. Fraser,2 Ravi S. Kamath,2 Julie Ahirner,4 Cynthia Kenyon5

To explore the role of mitochondrial activity in the aging process, we have lowered the activity of the electron transport chain and added a polyphosphate (ATP) synthase with RNA interference (RNAi) in Caenorhabditis elegans. These perturbations reduced body size and behavioral rates and extended adult life-span. Restoring messenger RNA to near-normal levels during adulthood did not elevate ATP levels and did not correct any of these phenotypes. Conversely, inhibiting respiratory-chain components during adulthood only did not reset behavioral rates and did not affect life-span. Thus, the developing animal appears to contain a regulatory system that monitors mitochondrial activity early in life and, in response, establishes rates of respiration, behavior, and aging that persist during adulthood.

During a systematic screen of a C. elegans chromosome I RNAi library (1, 2), we found that animals grown on bacteria expressing double-stranded RNA (dsRNA) encoding a component of the mitochondrial ATP synthase (ATP) synthase with RNA interference (RNAi) in Caenorhabditis elegans. These perturbations reduced body size and behavioral rates and extended adult life-span. Restoring messenger RNA to near-normal levels during adulthood did not elevate ATP levels and did not correct any of these phenotypes. Conversely, inhibiting respiratory-chain components during adulthood only did not reset behavioral rates and did not affect life-span. Thus, the developing animal appears to contain a regulatory system that monitors mitochondrial activity early in life and, in response, establishes rates of respiration, behavior, and aging that persist during adulthood.

The Photochemistry of Carotenoids

1. Department of Biochemistry and Biophysics, University of California, San Francisco, CA 94143–0448, USA.

2. Welcome/CRC Institute, University of Cambridge, Cambridge, United Kingdom.

3. Institute of Biological Sciences, Molecular and Cell Biology Laboratory, Institute of Biological Sciences, Molecular and Cell Biology Laboratory, La Jolla, CA 92037, USA.

4. To whom correspondence should be addressed. E-mail: ckennyon@biochem.ucsf.edu

5. Present address: The Salk Institute for Biological Studies, Molecular and Cell Biology Laboratory, La Jolla, CA 92037, USA.


7. Present address: Harvard University, Cambridge, MA 02115, USA.

8. To whom correspondence should be addressed. E-mail: ckennyon@biochem.ucsf.edu

9. Present address: ScienceOnline.

10. To whom correspondence should be addressed. E-mail: ckennyon@biochem.ucsf.edu

# R E P O R T S

"Reports" 20 DECEMBER 2002 VOL 298 SCIENCE www.sciencemag.org 2398
tion and ATP production decreases growth rate and body size, slows behavioral rates, and increases life-span.

The behavioral and longevity phenotypes of our RNAi-treated animals resemble those of isp-1 mutants, except that isp-1 mutants are not small (8). This suggests that our RNAi conditions are more severe. Mutations in the gene clk-1 also slow developmental rates and behaviors and extend life-span (9, 10). clk-1 mutants cannot produce ubiquinone and instead acquire it from the bacteria they eat (11, 12). However, the cause of the clk-1 phenotype is likely to be different from that of our RNAi-treated animals. ATP levels are normal or slightly elevated in clk-1 mutants (13), and surprisingly, we find that the longevity of clk-1 mutants (but not their reduced behavioral rates) depends on the presence of the somatic gonad (fig. S2, table S2).

In C. elegans, inhibiting either insulin/insulin-like growth factor–1 (IGF-1) signaling or germ line proliferation extends lifespan (14). Inhibiting insulin/IGF-1 signaling has been proposed to extend lifespan by reducing mitochondrial activity (8, 15). However, unlike the longevity produced by perturbing germ line or insulin/IGF-1 signaling, the longevity produced by these RNAi treatments were not dependent on the transcription factor daf-16 (Fig. 1B, table S1). In addition, the life-span extensions of our RNAi-treated animals were increased much further by daf-2 (insulin/IGF-1 receptor) mutations (Fig. 1C, table S1) and germ line ablation (fig. S2, table S1) (3). Finally, many long-lived insulin/IGF-1 mutants have normal growth and behavioral rates (16), and insulin/IGF-1 signaling mutants actually have much higher levels of ATP than normal (Fig. 2C) (13). Thus, these different perturbations appear to affect different life-span–regulatory pathways.

We next asked whether the rate of respiration acted in an ongoing manner throughout the life of the animal to affect behavioral rates and life-span or whether it was particularly important during a specific stage of life. First, we reduced respiratory-chain mRNA levels during development and then attempted to restore them during adulthood. To do this, we inhibited the RNAi machinery during adulthood by subjecting young adults treated with respiratory-chain RNAi from the time of hatching to Dicer (dcr-1) RNAi. The dcr-1 gene is required for RNAi (17, 18), and dcr-1 RNAi reduces RNAi activity (19). Shifting...
Fig. 3. Reduction of cco-1 (complex IV) expression during development is sufficient to lower the rate of metabolism during adulthood and increase life-span. To reduce cco-1 expression during development, we grew animals on bacteria expressing dcr-1 dsRNA from hatching until the first day of adulthood. To increase cco-1 expression in adulthood, we shifted these young adults to bacteria expressing dcr-1 dsRNA. (A) Concurrent measurement of cco-1 and dcr-1 mRNA levels by reverse transcriptase-polymerase chain reaction (RT-PCR) during day 1 until day 3 of adulthood. As dcr-1 mRNA levels fall, cco-1 mRNA levels rise. Shown are RT-PCR products from serial dilutions of total RNA isolated from animals grown on the RNAi vector, cco-1 dsRNA-expressing bacteria during development and then shifted to dcr-1 dsRNA bacteria, or cco-1 dsRNA-expressing bacteria during development and adulthood. (B) Concurrent ATP assays from the animals in (A). Increasing cco-1 mRNA levels during adulthood does not increase ATP levels. Black line, ATP levels of control animals grown on bacteria containing the RNAi vector; red line, ATP levels of animals grown on bacteria expressing cco-1 dsRNA during development and then shifted to dcr-1 RNAi bacteria during day 1 of adulthood. Blue line, ATP levels of animals grown continuously on bacteria expressing cco-1 dsRNA. (C) Red line, life-spans of animals grown on bacteria expressing cco-1 dsRNA during larval development and then shifted on day 1 of adulthood to bacteria expressing dcr-1 dsRNA for the remainder of their life. Blue line, life-span of animals grown on bacteria expressing cco-1 dsRNA during development and adulthood. Black line, life-span of animals grown on bacteria containing the RNAi vector only.

Fig. 4. Initiating RNAi of complex III (cyt-c1) or complex V (atp-3) during adulthood lowers ATP levels (A) but does not extend life-span (B). Animals were grown until the first day of adulthood on normal bacteria and then shifted to bacteria expressing cyt-c1 (complex III) or atp-3 (complex V) dsRNA. Control animals were shifted to bacteria containing the RNAi vector.
Complex IV (is the number of animals that produced fewer than 50 pumps per minute/total number of animals tested.

In eukaryotic cells, multidomain proteins are families, which consists of a range of receptors that share structural elements. Examples of multidomain proteins have been found in the LDL-R family, which consists of a range of receptors that share structural elements. The LDL-R itself is a surface glycoprotein that mediates cellular uptake of LDL (6). It has been proposed that its ectodomain consists of three regions (Fig. 1A). the NH2-terminally located ligand-binding region (composed of seven complement-like domains, each stabilized by three disulfide bonds and a calcium ion) (7, 8), the epidermal growth factor (EGF) precursor-like region (9, 10), and the abundantly O-glycosylated region. Structure determinations and in vitro folding studies of LDL-R fragments indicated a linear domain organization (Fig. 1A). This result suggests independent and sequential folding of the ligand-binding domains in the complete LDL-R (11, 12).

Protein folding in the endoplasmic reticulum (ER) is tightly linked with disulfide bond formation in the newly synthesized protein (13, 14). Whether non-native disulfide bonds are abundant or even essential in a folding pathway is still a matter of debate. Non-native bonds appear frequently in folding asays in vitro (15–17), but their occurrence in productive folding pathways in intact cells may be bypassed by the activity of protein..

Coordinated Nonvectorial Folding in a Newly Synthesized Multidomain Protein

Anemieke Jansens,* Esther van Duijn, Ineke Braakman†

The low-density lipoprotein receptor (LDL-R) is a typical example of a multidomain protein, for which in vivo folding is assumed to occur vectorially from the amino terminus to the carboxyl terminus. Using a pulse-chase approach in intact cells, we found instead that newly synthesized LDL-R molecules folded by way of “collapsed” intermediates that contained non-native disulfide bonds between distant cysteines. The most amino-terminal domain acquired its native conformation late in folding instead of during synthesis. Thus, productive LDL-R folding in a cell is not vectorial but is mostly posttranslational, and involves transient long-range non-native disulfide bonds that are isomerized into native short-range cysteine pairs.

In eukaryotic cells, multidomain proteins are thought to fold their domains independently and sequentially (1–3). Examples of multidomain proteins have been found in the LDL-R family, which consists of a range of receptors that share structural elements (4, 5). The LDL-R itself is a surface glycoprotein that mediates cellular uptake of LDL (6). It has been proposed that its ectodomain consists of three regions (Fig. 1A). the NH2-terminally located ligand-binding region (composed of seven complement-like domains, each stabilized by three disulfide bonds and a calcium ion) (7, 8), the epidermal growth factor (EGF) precursor-like region (9, 10), and the abundantly O-glycosylated region. Structure determinations and in vitro folding studies of LDL-R fragments indicated a linear domain organization (Fig. 1A). This result suggests independent and sequential folding of the ligand-binding domains in the complete LDL-R (11, 12).

Protein folding in the endoplasmic reticulum (ER) is tightly linked with disulfide bond formation in the newly synthesized protein (13, 14). Whether non-native disulfide bonds are abundant or even essential in a folding pathway is still a matter of debate. Non-native bonds appear frequently in folding asays in vitro (15–17), but their occurrence in productive folding pathways in intact cells may be bypassed by the activity of protein.

**References and Notes**

3. Materials and methods are available as supporting

**Supporting Online Material**

www.sciencemag.org/cgi/content/full/1077780/DC1

Materials and Methods

Figs. S1 and S2

Tables S1 and S2

27 August 2002; accepted 14 November 2002

Published online 5 December 2002; 10.1126/science.1077780

Include this information when citing this paper.

Department of Bio-Organic Chemistry 1, Blijvoet Center for Biomolecular Research, University of Utrecht, Padualaan 8, 3584 CH Utrecht, Netherlands.

*Present address: Biological Sciences, Stanford University, 325 Serra Mall, Stanford, CA 94305, USA.

†To whom correspondence should be addressed. E-mail: ibbraakman@chem.uu.nl

---

**Table 1.** Pumping rates of larvae and adults treated with respiratory chain RNAi.

<table>
<thead>
<tr>
<th>RNAi treatment</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feeding rate (pumps per minute)*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vector</td>
<td>118 ± 15</td>
<td>140 ± 21</td>
<td>145 ± 24</td>
<td>131 ± 49</td>
</tr>
<tr>
<td>(0/8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complex III (cyc-1)</td>
<td>97 ± 21</td>
<td>89 ± 12</td>
<td>84 ± 12</td>
<td>75 ± 12</td>
</tr>
<tr>
<td>(0/10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complex III (cyc-1)</td>
<td>79 ± 62</td>
<td>112 ± 80</td>
<td>80 ± 59</td>
<td>69 ± 45</td>
</tr>
<tr>
<td>adult only</td>
<td>(5/12)</td>
<td>(3/12)</td>
<td>(4/12)</td>
<td>(4/12)</td>
</tr>
<tr>
<td>Complex V (atp-3)</td>
<td>98 ± 15</td>
<td>87 ± 10</td>
<td>80 ± 9</td>
<td>75 ± 8</td>
</tr>
<tr>
<td>larval and adult</td>
<td>(0/10)</td>
<td>(0/10)</td>
<td>(0/10)</td>
<td></td>
</tr>
<tr>
<td>Complex V (atp-3)</td>
<td>88 ± 80</td>
<td>98 ± 64</td>
<td>83 ± 59</td>
<td>89 ± 54</td>
</tr>
<tr>
<td>adult only</td>
<td>(6/14)</td>
<td>(5/14)</td>
<td>(4/14)</td>
<td>(4/14)</td>
</tr>
</tbody>
</table>

*Mean ± SD. The number of pharyngeal pumps observed in an adult animal in 1 min at 25°C. Number in parentheses is the number of animals that produced fewer than 50 pumps per minute/total number of animals tested.