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Genetics

Influence of TOR kinase on lifespan in *C. elegans*

The group of enzymes known as TOR (for 'target of rapamycin') kinases regulates cell growth and proliferation in response to nutrients and hormone-dependent mitogenic signals[1,2]. Here we show that TOR deficiency in the nematode *Caenorhabditis elegans* more than doubles its natural lifespan. This new function for TOR signalling in ageing control may represent a link between nutrition, metabolism and longevity.

In *C. elegans*, the absence of LET-363/TOR activity causes developmental arrest at the L3 larval stage[3]. We examined nematodes bred as *let-363/CeTor* genetic null mutants and nematodes that had been depleted of TOR by using RNA interference to block *let-363* expression (termed *let-363*-(RNAi) worms), and found that these animals had a strikingly extended mean lifespan (Fig. 1a, squares and triangles, respectively). At 25.5 °C, the mean lifetime was 25 days in *let-363* mutants compared with 10 days in wild-type animals. This is all the more intriguing in light of the fact that TOR-deficient worms existed as arrested L3 larvae. In comparison, L3 larval arrest induced by starvation persisted for only 14 days on average in wild-type animals (Fig. 1a, diamonds).

Strong inhibition of mitochondrial respiration also arrests development at the L3 stage, whereas weaker inhibition permits growth to adulthood and extends adult lifespan, but only if it occurs during larval development[4]. In contrast, treatment with *let-363* double-stranded RNA starting from the first day of adulthood lengthens lifespan to a comparable extent when RNAi treatment is initiated at hatching (Fig. 1a, open triangles). This indicates that TOR has a role in ageing control during adulthood and that the long-lived phenotype of *let-363*-(RNAi) adults cannot be explained by reduced mitochondrial activity.

Longevity in *C. elegans* is controlled hormonally by a conserved signalling pathway that involves insulin and insulin-like growth factor (IGF)[5,6]. Mutants with reduced DAF-2/IGF signalling activity live twice as long as the wild type[5,6]. The DAF-2/IGF cascade also acts during adulthood to influence ageing[7]. The remarkable similarity in the developmental stage at which ageing rate is affected, and our finding that the extended lifespan of *daf-2(e1370)* mutants is not increased further by treatment with *let-363* RNAi (results not shown) — as it is with RNAi blocking expression of respiratory-chain components[4] — raise the possibility that TOR and the DAF-2/IGF pathway are related in controlling lifespan.

This idea is compatible with results indicating that the insulin/IGF cascade regulates protein synthesis and cell growth in mammals and *Drosophila* through the activity of nutrient-sensing TOR (reviewed in refs 1, 2, 8). We have also noted that *let-363*-(RNAi) animals share certain features of the pleiotropic Daf-2(−) phenotype, such as lipid accumulation mainly in intestinal cells[9] (Fig. 1b), as well as reduced fertility[10] (mean brood sizes: *let-363*-(RNAi) adults, 68 ± 6.4; wild type, 191 ± 14.5) and reduced viability[10] (embryonic/early larval arrest: *let-363*-(RNAi), 40.3%; wild type, 5.4%).

Strong mutations in DAF-2/IGF signalling cause a long-lived phenotype, together with a state of developmental diapause known as dauer that is triggered by starvation and crowding in the wild type[11]. According to our results (not shown), *let-363(h111)* animals bearing the thermosensitive *daf-2(e1370ts)* mutation were able to form dauers at the restrictive temperature. Furthermore, *let-363*-(RNAi) enhanced dauer formation in *daf-2(e1370)* animals. At 20 °C, only 4.6% (29 out of 630) of *daf-2(e1370)* mutants entered into the dauer stage, compared with 17.9% (146 out of 817) of *daf-2(e1370); let-363*-(RNAi) animals (results not shown). This indicates a genetic interaction between *let-363/CeTor* and *daf-2*. These results show that in *C. elegans* the TOR and DAF-2/IGF signalling pathways could be related in controlling ageing, metabolism and reproductive growth.

Lifespan extension in *daf-2(e1370)* mutants requires the activity of the forkhead transcription factor DAF-16 (refs 5, 6). Mutations in *daf-16*, however, do not suppress the long-lived phenotype of *let-363*-(RNAi) worms (Fig. 1a, filled triangles), indicating that TOR may be acting downstream or independently of DAF-16, and that it is interacting with the insulin endocrine system. Although the detailed signalling connections require clarification, our findings point to TOR as a possible mediator of lifespan regulation by insulin signalling and nutrient sensing.

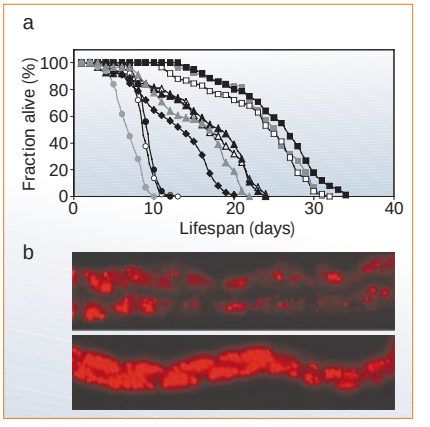

Figure 1 TOR deficiency in the nematode *Caenorhabditis elegans*. **a,** Lifespan of TOR-deficient worms compared with the wild type at 25.5 °C: wild type (filled circles); *daf-16(mg50)* (open circles); *dpy-5(e61) unc-13(e450)* double mutant (shaded circles); TOR-deficient triple mutants *let-363(h114) dpy-5(e61) unc-13(e450)* (open squares), *let-363(h111) dpy-5(e61) unc-13(e450)* (filled squares) and *let-363(h131) dpy-5(e61) unc-13(e450)* (shaded squares); *let-363*-RNAi-treated worms from hatching (open triangles) or from the first day of adulthood (shaded triangles); *let-363*-RNAi-treated *daf-16(mg50)* worms (filled triangles); starving-arrested wild-type L3 larvae (filled diamonds). Disruption of TOR by RNAi (triangles) seems to be incomplete, as lifespan is not extended as much as in *let-363* mutants (squares). **b,** Nile Red staining of lipid droplets in a wild-type L3 larva (top) and an L3 larva arrested by *let-363*-(RNAi) treatment (bottom). Images were obtained with the same exposure time.

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show that 99% of the variation in their basal rates of metabolism can be accounted for by interspecific variation in body mass, food habits and altitudinal distribution. These findings, which are derived from 31% of the species and 53% of the genera in this family, give the most complete picture of the standard energetics of any diverse family of birds.

Basal rate, regarded as the standard rate of metabolism in endotherms, conforms to a power function that increases with body mass and varies with food habits, climate, body composition, a continental or island distribution, and possibly other factors[1]. Individual and population growth rates[2], reproductive rates[3] and energy expenditure in the field, at least in mammals[1,4–7], correlate with variation in basal rate.

Among passerine birds, one of the most distinctive families is the Paradisaeidae, which is found principally in New Guinea. This family is noted for the elaborate plumage of most males, widespread use of lek polygyny, extended longevity, low reproductive rate and diverse food habits, which vary from strict frugivory to extensive insectivory[8].

In an analysis of covariance, the $\log_{10}$ basal rates of 13 species of Paradisaeidae, measured in rainforest habitat at the Technological University in Lae, Papua New Guinea, simultaneously correlated with $\log_{10}$ body mass ($F_{1,8} = 755.09$, $P < 0.0001$), food habits ($F_{2,8} = 28.78$, $P = 0.0002$) and the altitude at which these species live ($F_{1,8} = 7.98$, $P = 0.023$). Predicted basal rates of paradisaeids (in ml O_2 per hour) are described by

$$V^{Y}_{O_2} = 3.35(FA)g^{0.879} \qquad (1)$$

where F is a non-dimensional coefficient for food habits, A is a non-dimensional coefficient for altitude, and g is body mass in grams. The coefficients are antilogarithms of the intercepts of the curve of $\log_{10}$ basal rate on $\log_{10}$ body mass.

In this case, F is 1.00 for omnivores ($n = 5$), 0.906 for insectivores ($n = 3$) and 0.757 for frugivores ($n = 5$); A is 1.00 for species that live at altitudes greater than 1,000 m ($n = 9$) and 0.906 for species restricted to lower altitudes ($n = 4$). Equation (1) accounts for 99.0% of the variation in basal rate in this family ($F_{1,11} = 1{,}114.24$, $P < 0.0001$), which assures that all studied species conform to a single relationship, irrespective of body mass, food habits or the altitude at which they live (Fig. 1).

In birds of paradise that preferentially eat fruit and live at altitudes of less than 1,000 m, the combined coefficient in equation (1) is $3.35 \times 0.757 \times 0.906 = 2.30$, whereas in high-altitude omnivores it is $3.35 \times 1.00 \times 1.00 = 3.35$, or 1.46 times that of the low-altitude frugivores. Fruit-eating specialists have basal rates that are 79.4% of the basal rates of species that eat insects as more than 10% of their diet, and species that live at altitudes below 1,000 m have basal rates that are 90.6% of those that live at higher altitudes. Other combinations of body mass, food habits and altitude account for the observed basal rates of the remaining species. Body mass alone accounts for 91.7% of the variation in basal rate; 97.5% of this variation is accounted for by the combination of body mass and food habits.

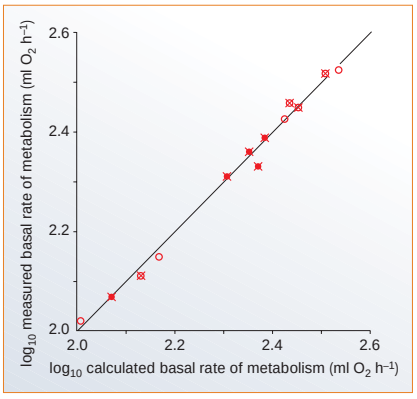

Figure 1 $\log_{10}$ basal rates of metabolism measured in birds of paradise as a function of $\log_{10}$ basal metabolic rates predicted by equation (1). Food habits: hollow circles, insect and mixed diet; filled circles, fruit diet. Crosses indicate birds living at altitudes above 1,000 metres.

A low basal metabolic rate in frugivorous birds is also seen in manakins (Pipridae)[9], pigeons (Columbidae)[10], toucans (Ramphastidae)[11] and a hornbill (Bucerotidae)[11], although frugivorous passerines have higher basal rates than frugivorous non-passerines, contrary to the view that there is no difference in the basal rates of non-passerines and passerines[12]. An examination of the evolutionary relationships within the Paradisaeidae indicates that frugivory[13] and low basal rate are plesiomorphic conditions in this family. The evolution of high basal rates in the studied species occurred at least three times in relation to movement into higher altitudes, and twice in relation to the adoption of an omnivorous or insectivorous diet. The use of phylogeny as the 'cause' for the evolution of character states[7,12] is therefore doubtful.

The remarkable plumage of males in dimorphic species of this family seem to entail very high costs during synthesis: rates of metabolism increase by at least 53% during moulting in male king birds of paradise (*Cicinnurus regius*). However, brightly coloured males in species with sexually dimorphic plumage do not have higher basal rates than dull-coloured females of the same species ($F_{2,4} = 0.46$, $P = 0.66$), and sexually dimorphic species do not have higher basal rates than sexually monomorphic species ($F_{1,8} = 0.87$, $P = 0.37$). Furthermore, species that use lek displays do not have higher basal rates ($F_{1,10} = 4.45$, $P = 0.079$) than those that use solitary displays.

The behaviour and ecology of the Paradisaeidae has been extensively studied, but the energetics of these species have not been evaluated until now. The analysis of covariance that I used for this task is superior to the conventional phylogenetic-contrasts approach.

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COMMUNICATIONS ARISING

Geochronology

Dating of the Herto hominin fossils

The age of Pleistocene *Homo sapiens* fossils and archaeological material from the Bouri Formation in the Middle Awash region of Ethiopia, discovered in 1997 by White and colleagues[1,2], has been constrained to between 160 ± 2 and 154 ± 7 kyr on the basis of isotopic dating and stratigraphic and geochemical evidence[2]. However, our analysis of their stratigraphic and geochronological data indicate that, although the estimated maximum age (160 ± 2 kyr) is valid, the minimum age (154 ± 7 kyr) is doubtful. These important discoveries[3] may therefore be distinctly younger than reported[1,2].

The fossils and archaeological remains occur in volcaniclastic sandstones and gravel deposits of the older part of the Upper Herto Member of the Bouri Formation. The lower boundary of this member is formed by a widespread erosional surface. Immediately below this surface is a bentonite tuff (MA97-1, 2) which has been isotopically dated to 260 ± 16 kyr. Two pumices and two obsidian clasts from the fossiliferous deposits above the erosional surface provide a relatively narrow age range close to 160 kyr, which indicates a maximum age for the deposit, but it cannot necessarily be inferred that it is "close to the actual time of deposition of this fossiliferous unit"[2].

The main problem in dating the fossiliferous horizon is how to establish a younger age limit. The Upper Herto Member is capped by the stratigraphically important Waidedo Vitric Tuff (WAVT, MA92-1). Unfortunately, this has proved difficult to date isotopically because of contamination by older feldspar