

**Dietary restriction increases
locomotor activity of the swimming gait in
*Caenorhabditis elegans***

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List of Abbreviations

µg	microgram
µl	microliter
A	alanine
aa	amino acids
ACh	acetylcholine
AIY	amphid interneuron
AL	<i>ad libitum</i>
AMP	5' adenosine monophosphate
AMPK	5' adenosine monophosphate-activated protein kinase
ANOVA	analysis of variance
approx.	approximately
AU	arbitrary unit
bb	body bends
BBCF	body bending crawling frequency
BBSF	body bending swimming frequency
BCA	bicinchoninic acid
BCAA	branched-chain amino acids
BSA	bovine serum albumin
<i>C.elegans</i>	<i>Caenorhabditis elegans</i>
CMC	carboxy methylcellulose
COPAS	Complex Object Parametric Analyzer and Sorter
CR	caloric restriction
D	aspartic acid
DAG	diacylglycerol
<i>DMRT3</i>	doublesex and mab-3 related transcription factor 3
DR	dietary restriction
E	glutamic acid
<i>E.coli</i>	<i>Escherichia coli</i>
e.g.	<i>exempli gratia</i> ; for example
Ext	extinction
F	phenylalanine
FMRFamide	phenylalanin-methionine-arginine-phenylalanine neuropeptide
G	glycine
H	histidine
h	hour
Hz	Hertz, here body bends per second
I	isoleucine
i.e.	<i>id est</i>
IGF	insulin-like growth factor 1
IP3	inositol (1,4,5)-trisphosphate
K	lysine
L	leucine
l	liter
L4	larval stage 4
M	methionine
mg	milligram
min	minute

ml	milliliter
mm	millimeter
mM	millimol
mPa	millipascal
MWT	Multi-Worm Tracker
NAD	nicotinamide adenine dinucleotid
NADH	nicotinamide adenine dinucleotid (reduced form)
NGM	Nematode Growth Medium
nm	nanometer
P	proline
PA	phosphatic acid
PIP2	phosphatidylinositol (4,5)-bisphosphate
ppm	parts per million
R	arginine
RF	re-feeding
rpm	rounds per minute
RT	room temperature
S	serine
s	second
SD	standard deviation
SIRT	NAD-dependent sirtuin
Starv	starvation
T	threonine
TOST	two one-sided test
TOF	time of flight
TOR	target of rapamycin
TORC1	target of rapamycin complex 1
TORC2	target of rapamycin complex 2
V	valine
vs.	<i>versus</i>
W	tryptophan
wt	wild type
Y	tyrosine

Summary

Animals with a limb system as well as invertebrates need a well-developed locomotion system for foraging, mating and to survive in response to environmental changes. They are able to switch between alternative forms of locomotion, referred to as gaits, to adapt to different environments. The nematode *Caenorhabditis elegans* (*C.elegans*) uses two distinct functional locomotory gaits, swimming in liquids and crawling on dense gels. These gaits differ in their kinematics of the generated undulations and their pattern of muscle activity. Crawling is energetically more efficient than swimming. While the locomotion behaviour of *C. elegans* is well described, gait specific locomotion responses are less examined. In this study, the functional activity of the crawling and swimming gait, respectively, were analysed by means of phenotypic analysis of *C. elegans* wild type and mutant strains.

Results of this study demonstrate that wild type worms respond to dietary restriction (DR) with remarkably accelerated body bending swimming frequency (BBSF) of 53 % increase compared to *ad libitum* (AL) fed animals. Simultaneously, the body size, protein content and muscular strength, which was determined using analysis of swimming in viscous liquids, was reduced in dietary restricted animals. Whereas the resulting speed of swimming was also increased in dietary restricted worms. Remarkably, the DR induced acceleration of swimming was found to be gait specific since the functional activity of the crawling gait was decreased in response to DR. Moreover, in contrast to the crawling gait, the activity of the swimming gait is independent of bacterial food being present or lacking in the assay medium.

In order to identify pathways, which might be involved in mediating the DR response of increased BBSF, several candidate mutant strains were analysed. This approach revealed that the canonical synaptic G-protein signalling network $G\alpha_o$ - $G\alpha_q$, which regulates acetylcholine release from motoneurons, is required for the DR induced acceleration of BBSF. In particular, the octopamine receptor SER-3 and GBP-2, a central regulator of G-protein signalling, seems to be of central importance for the swimming response to DR. In addition, the TOR (target of rapamycin) and AMPK (5' adenosine monophosphate-activated protein kinase) pathway, which sense intracellular amino acids and energy status, respectively, might contribute to the response. In conclusion, this study identified evolutionary conserved pathways, which are important for acceleration of swimming activity in response to limited food supply

in the nematode *C. elegans*. This adaptation might be a survival strategy of food deprived nematodes exposed to liquids to direct their motion toward favourable conditions.

Zusammenfassung

Nahezu alle Lebewesen, ob mit oder ohne Gliedmaße, benötigen eine gut entwickelte Motorik um Bewegungsabläufe für die Futtersuche und Paarung zu steuern, um auf Veränderungen der Umwelt zu reagieren und damit das Überleben zu sichern. Die meisten Tiere sind in der Lage zwischen verschiedenen Bewegungsformen, auch als Gangarten bezeichnet, zu wechseln und können sich auf diese Weise an verschiedene Umwelteinflüsse anpassen. Die Nematode *Caenorhabditis elegans* (*C. elegans*) verwendet zwei verschiedene Gangarten, nämlich das Schwimmen in Flüssigkeiten und das Kriechen über dichte Gele. Diese beiden Gangarten unterscheiden sich in der Kinematik der erzeugten Wellenformen und dem Muster der Muskelaktivität, wobei kriechen energetisch effizienter ist als schwimmen. Während das Fortbewegungsverhalten von *C.elegans* bereits ausführlich beschrieben wurde, sind Gangart spezifische Reaktionen weniger gut untersucht. In der vorliegenden Studie wurde die funktionelle Aktivität der Kriech- und Schwimmgangart mittels phenotypischer Analyse des *C.elegans* Wildtyp Stammes sowie verschiedener *C.elegans* Mutanten untersucht.

Die Ergebnisse dieser Studie zeigen, dass Wildtyp Nematoden unter diätischer Restriktion (DR) ihre "body bending swimming frequency" (BBSF, "Körperbeugungs-Schwimmfrequenz") um 53 % im Vergleich zu *ad libitum* (AL) ernährten Nematoden beschleunigen. Während die Körpergröße, der Proteingehalt und die Muskelkraft, die durch Analyse des Schwimmens in viskosen Flüssigkeiten bestimmt wurde, in restriktiv ernährten Nematoden reduziert ist, war jedoch die resultierende Schwimgeschwindigkeit in diesen restriktiv ernährten Nematoden erhöht. Bemerkenswerterweise stellte sich heraus, dass die durch DR induzierte Beschleunigung des Schwimmens Gangart spezifisch ist, da die funktionelle Aktivität des Kriechens als Reaktion auf DR verringert war. Darüber hinaus ist die Aktivität des Schwimgangs, im Gegensatz zum Kriechgang, unabhängig von der An- oder Abwesenheit von bakterieller Nahrung im Untersuchungsmedium.

Um Übertragungs- bzw. Signalwege zu identifizieren, die möglicherweise die durch DR induzierte erhöhte BBSF vermitteln, wurden verschiedene *C.elegans* Mutanten untersucht. Dieser Ansatz zeigte, dass das synaptische G-Protein Signalnetzwerk $G\alpha_o$ - $G\alpha_q$, das die Acetylcholinausschüttung an Motoneuronen reguliert, für die DR induzierte Beschleunigung der BBSF benötigt wird. Insbesondere der

Octopaminrezeptor SER-3 und GBP-2, ein Hauptregulator der G-Protein gekoppelten Signalwege, scheinen von zentraler Bedeutung für die Schwimmreaktion auf DR zu sein. Daneben tragen möglicherweise auch der TOR (target of rapamycin) und der AMPK (AMP-aktivierte Proteinkinase) Signalweg, die den intrazellulären Aminosäure-beziehungsweise Energiestatus wahrnehmen, zu dieser Reaktion bei.

Zusammenfassend identifizierte diese Studie evolutionär konservierte Signalwege, die bedeutend für die Beschleunigung der Schwimmaktivität als Reaktion auf limitiertes Nahrungsangebot in der Nematode *C. elegans* sind. Diese Anpassung könnte eine Überlebensstrategie der restriktiv ernährten und Flüssigkeiten ausgesetzten Nematoden darstellen, um sich unmittelbar aus dieser Lage zu befreien und sich möglichst schnell zu einer vorteilhafteren Umgebung zu bewegen.

1. Introduction

1.1. Model organism *Caenorhabditis elegans*

Caenorhabditis elegans (*C. elegans*) has been established as a model organism by Sydney Brenner in 1974 [1] to study fundamental biological processes. The small, soil-living nematode belongs to the family of *Rhabditidae* and feeds on bacteria (e.g. *E. coli*) at decomposing organic material. It prefers moderate temperatures between 15°C and 25°C. *C. elegans* has two sexes, the self-reproducing hermaphrodites (XX), which self-fertilize their oocytes, and males (X0), which appear spontaneously with a rate of 0.1 % in a population. Hermaphrodites are able to produce 300 genetically identical progeny in their reproductive period. Once an egg is laid, it takes about 72 h at 20°C until the nematode reaches adulthood and egg laying begins again. During development the worms go through four larval stages, each marked by molting (**figure 1**). The average lifespan of *C. elegans* is about 2-3 weeks [2-4]. However, early larvae can enter a dauer state in times of unfavourable conditions like diminishing food source, overcrowding or increasing temperature. Dauer larvae are able to survive a few months in this arrested state and turn into L4 larvae if environmental conditions improve [5].

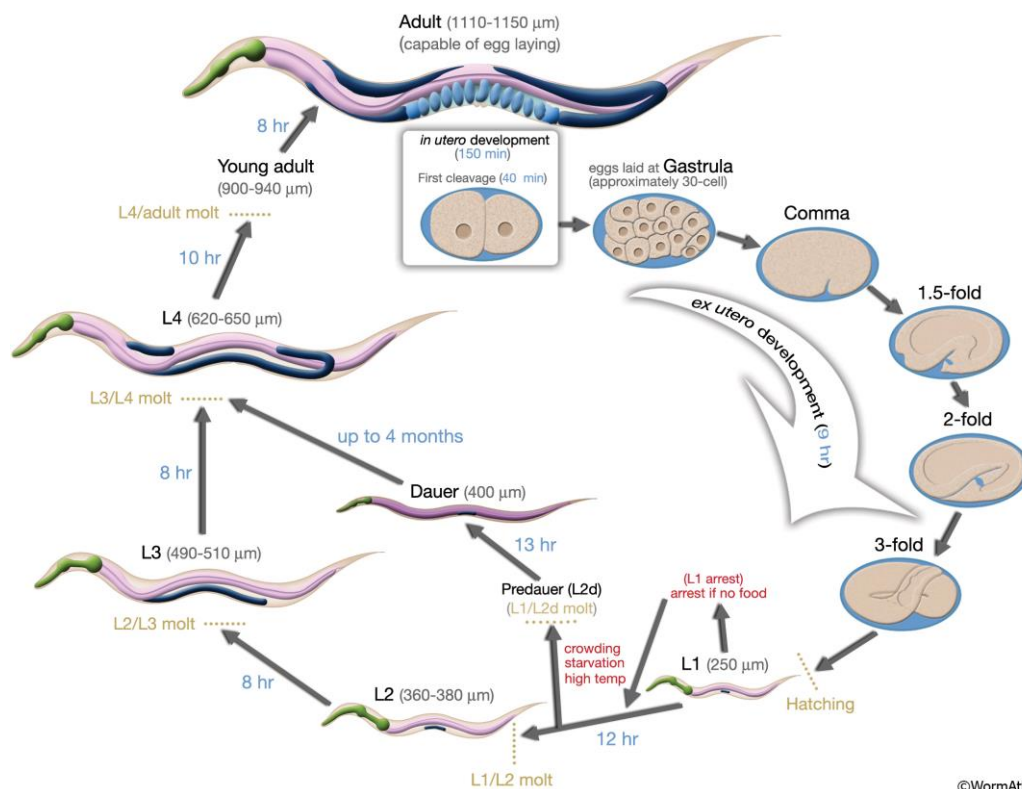


Figure 1. Life cycle of *C.elegans*. Provided by WormAtlas, www.wormatlas.org, 2012

As a roundworm, *C. elegans* has a cylindrical body shape that is un-segmented. The pseudocoelom separates the outer body tube, mainly defined by the cuticle, hypodermis, excretory system, neurons and body wall muscles, from the inner tube, consisting of the pharynx, intestine and gonads (**figure 2**) [6].

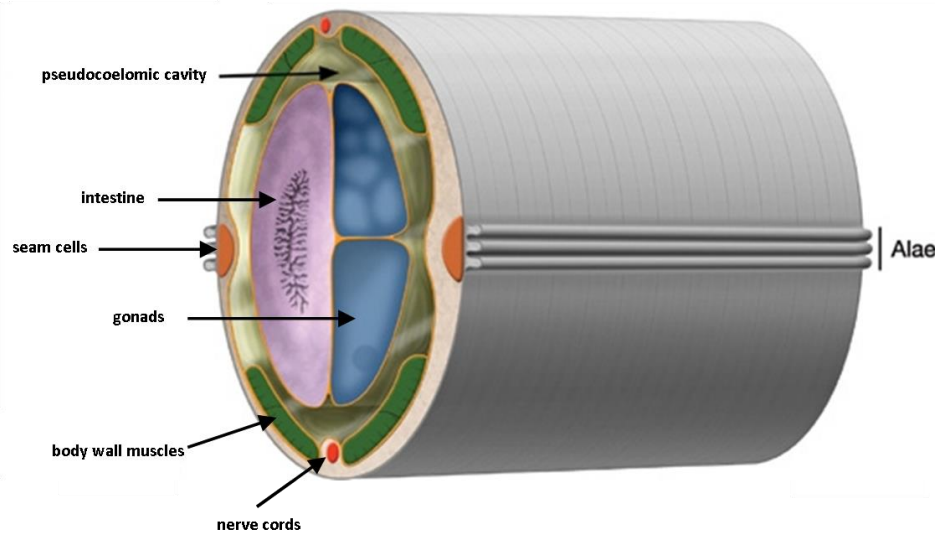


Figure 2. Body shape of posterior body region of *C.elegans*. Modified from WormAtlas, www.wormatlas.org, 2012

C. elegans turned out to be an excellent organism to study a wide range of biological processes. Its body size is small (≈ 1 mm), the generation time is short, the breed size is large and the costs for maintenance on *E. coli* seeded agar plates are low. Additionally, the worm exhibits a simple nervous system and the genetic homogeneity of the homozygous hermaphrodites and the constancy of 959 somatic cells in adult hermaphrodites are convincing. Furthermore, the whole genome of *C. elegans* has been sequenced in 1998, as the first multi-cellular organism [7]. Mutant strains can be simply produced, stored and analysed. *C. elegans* is also one of the few multi-cellular organisms, which can be stored over a long time by freezing in glycerol stocks at -80°C [8].

1.2. Evolutionary conserved adaptations and mechanisms in response to dietary restriction

Living organisms must be able to react to changing environmental conditions. In particular, in times when food is scarce it is essential that organisms have adaptive mechanisms, in order to ensure survival. A wide range of physiological responses have been found in food deprived organisms. The metabolism alters under food deprivation by shifting from carbohydrate towards fat metabolism, mobilizing fat stores, used as energy reserves. As a result, the body composition as well as gene expression is altered, as found in mice, worms and flies [9-15]. To conserve energy in times of prolonged food restriction, growth is reduced and reproductive rate decreased [16-21]. Food deprived animals also showed an enhanced stress resistance to oxidative and thermal stress, which further increase the chance of survival [22, 23]. In almost all organisms [19, 20, 24], food restriction extends lifespan by delayed aging as a consequence of altered metabolism and signalling pathways. Different experimental set-ups are used to investigate adaptive mechanisms in response to food restriction: starvation, the total removal of food; caloric restriction (CR), reduction of energy without reduction of micro-nutrients; dietary restriction (DR), a simultaneous reduction of energy and all nutrients without malnutrition [10, 25]. Although these protocols differ markedly, common responses were identified (for a review: Speakman, 2011 [10]). Here, we used the term dietary restriction (DR) as a synonym for regimes with reduced food intake or food availability.

Most of the research, investigating adaptive responses to DR, is focused on the topic of longevity and understanding of the underlying molecular and genetic mechanisms. Glucose and insulin levels are reduced under DR and a decrease of insulin/insulin-like growth factor 1 (IGF-1) signalling has been shown to extend lifespan in an evolutionary conserved way [26, 27]. IGF-1 signalling also interacts with the TOR (target of rapamycin) signalling pathway, which activity is decreased during DR [28, 29]. TOR kinase pathway is known to maintain cell functions like metabolism, proliferation and autophagy and is also induced by sensing the intracellular energy and nutrient status or amino acids [30-34]. DR increases cellular 5' adenosine monophosphate (AMP) levels that are sensed by AMP-activated-protein-kinase (AMPK), which for their part also regulates TOR activity and affects carbohydrate and lipid metabolism [35-37]. In worms, autophagy was found to be critical for survival

under starvation condition [38]. Autophagy processes are negatively regulated by TOR, but activated by NAD⁺-dependent deacetylases, called sirtuins (SIRT). SIRT proteins act as metabolic sensors of NAD⁺/NADH ratio and are necessary for DR induced increase in lifespan [39, 40]. In mammals, SIRT-1 regulates gluconeogenesis [41-44]. Recently, it has been reported that p53, a tumour suppressor protein, might be a link between SIRT-1 and TOR pathway and has an impact on AMPK and IGF-1 pathway [45] (**figure 3**). However, IGF-1, TOR, AMPK, autophagy and SIRT signalling pathways build a cross-linked network that has been found in yeast, worms, flies and mammals, suggesting an evolutionary conserved role of these pathways.

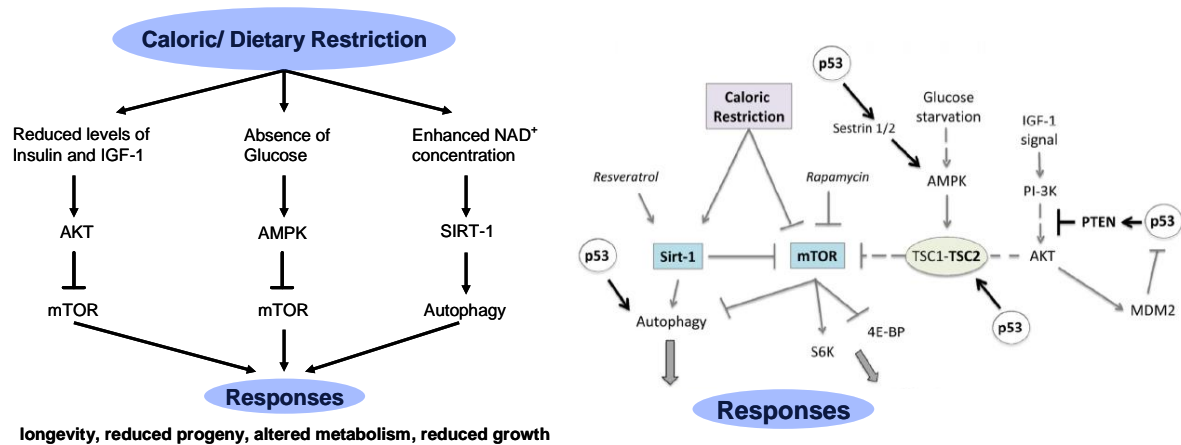


Figure 3. Molecular mechanisms of caloric restriction extending lifespan. Modified from P. Tucci, 2012 [45].

1.3. Adaptations and mechanisms of locomotion in response to different environments

A well developed locomotion system is crucial for animals from widely diverse taxa to react to environmental changes and to ensure survival. Locomotion is usually coordinated by a neuronal system that generates rhythmic neuromuscular activity, which can be either symmetrical or asymmetrical [46]. An adaptation to different environments is often accompanied by the use of qualitatively distinct types of locomotion, referred to as locomotory gaits. For instance, horses and other quadrupeds can alternate between the symmetrical gaits of walking, trotting or pace and the asymmetrical gaits gallop or canter [47]. Recently, *Dmrt3*, a master regulator for differentiation of a subclass of interneurons, was found to be required for coordinated locomotion in mice and mutations in *Dmrt3* affect special gaits in horses [48]. Gait switches are often triggered by environmental influences, like escape from predators, temperature and salinity shifts or external mechanical load of the surrounding medium, and food availability. Shrimps react to changes of the surrounding temperature or salinity with altered locomotion [49, 50]. Fishes adjust their locomotion pattern and speed of movement to the mechanical load of the surrounding substrate. Besides swimming, anguilliform fishes are able of subsurface locomotion and burrowing [51]. The small sandfish lizard can even switch from crawling on the surface to swimming through the sand if threatened [52].

C. elegans is an excellent model organism to study adaptive locomotion behaviour, because the nematodes can crawl through soil, glide on smooth surfaces and swim in liquids. The kinematic of *C. elegans* locomotion is well described and shows two distinct functional locomotory gaits: swimming and crawling. Since the Reynolds number of *C. elegans* swimming is less than unity, worms need a lot of energy to swim straight forward [53-55]. The swimming gait is characterized by c-shaped body bending with higher bending frequency and longer bending amplitude compared to the crawling gait (**figure 4**) [53, 56].

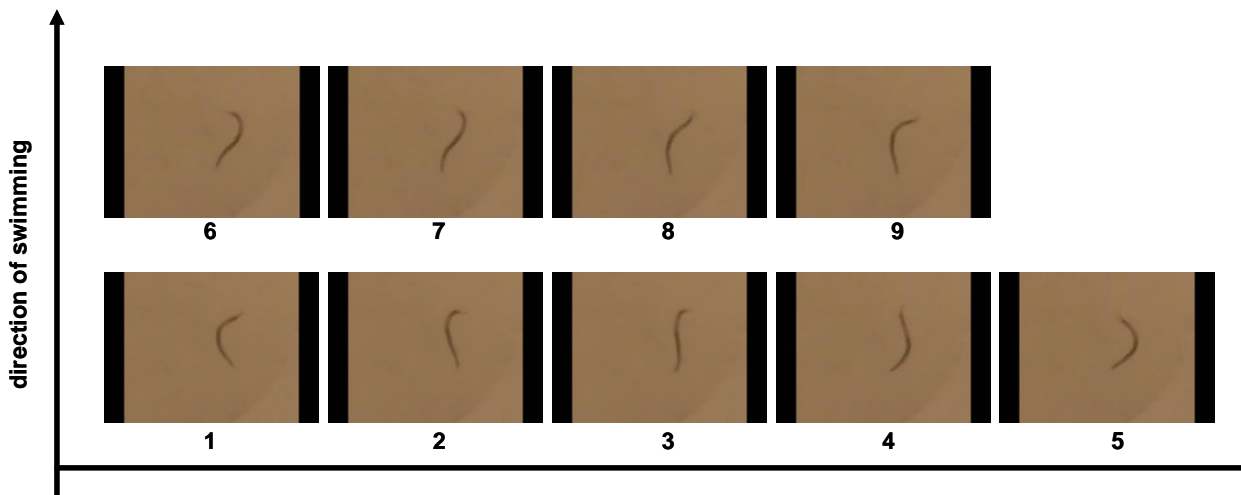


Figure 4. Sinusoidal swimming undulations of *C.elegans*, swimming in M9 buffer. Images 1-9 define one fully performed body bend. The graph was created after Gray and Lissmann, 1964 [53].

In which the crawling gait is characterized by sinusoidal undulations with s-shaped body bends of shorter wavelength and lower bending frequency (**figure 5**) [53, 56]. The undulations are achieved by reciprocal contractions of the longitudinal body wall muscles to dorso-ventral bends [53, 54].

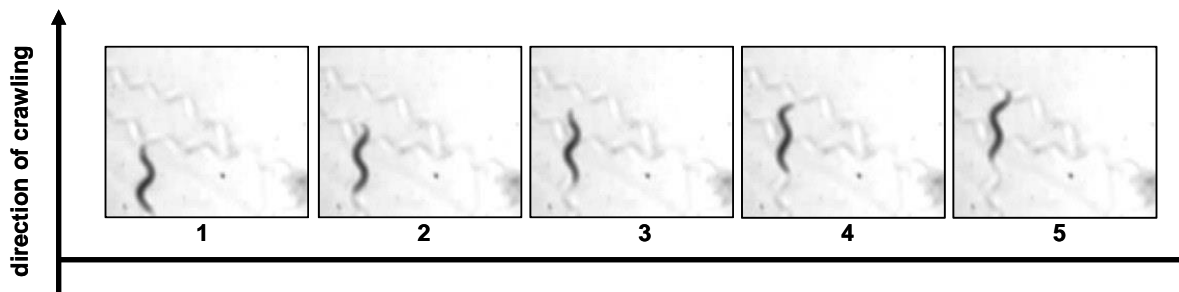


Figure 5. Typically s-shaped crawling undulations of *C.elegans*, crawling at bacteria containing agar plate. The graph was created after Gray and Lissmann, 1964 [53].

The distinct locomotion patterns in *C. elegans* are regulated by serotonin and dopamine signalling. In which the transition from crawling to swimming results from serotonergic signalling, whereas the transition from swimming to crawling is mediated by dopaminergic signalling [57]. Recently, dopamine has shown to be required for precise modulation of locomotor activity and speed at crawling gait. Distinct patterns of neuromuscular activity were also found for both gaits [56], whereas it is generally assumed that excitation of muscles, inducing locomotion, is mediated by interaction

of the activating EGL-30 ($G\alpha_q$) and inhibiting GOA-1 ($G\alpha_o$) signalling network. The EGL-30 pathway activates phospholipase C β (EGL-8), which is responsible for degradation of phosphatidylinositol (4,5)-bisphosphate (PIP₂) to inositol (1,4,5)-trisphosphate (IP₃) and diacylglycerol (DAG) [58]. DAG is bound by UNC-13, required for synaptic vesicle priming and synaptic transmission, which leads to acetylcholine (ACh) release from motor neurons, inducing muscle contraction. An inhibition of synaptic transmission and thereby locomotion is provided by serotonin. Serotonin activates GOA-1, affecting the diacylglycerol kinase, DGK-1, which degrades DAG to phosphatidic acid (PA) and depletes the DAG levels. A central regulator, mediating between GOA-1 and EGL-30 signalling network is GBP-2, a G-protein β -subunit [59-64]. $G\alpha_q$ signalling is further regulated by $G\alpha_s$ signalling pathway, which regulates locomotion response to DAG. In *C.elegans* $G\alpha_s$ signalling occurs via RIC-8, a receptor-independent G α guanine nucleotide exchange factor that activates GSA-1, a G-protein α -subunit. By activation of ACY-1, an adenylyl cyclase, the cAMP levels increase and the protein kinase A KIN-1 is activated, which affects UNC-13 mediated vesicle priming of DAG (figure 6) [59].

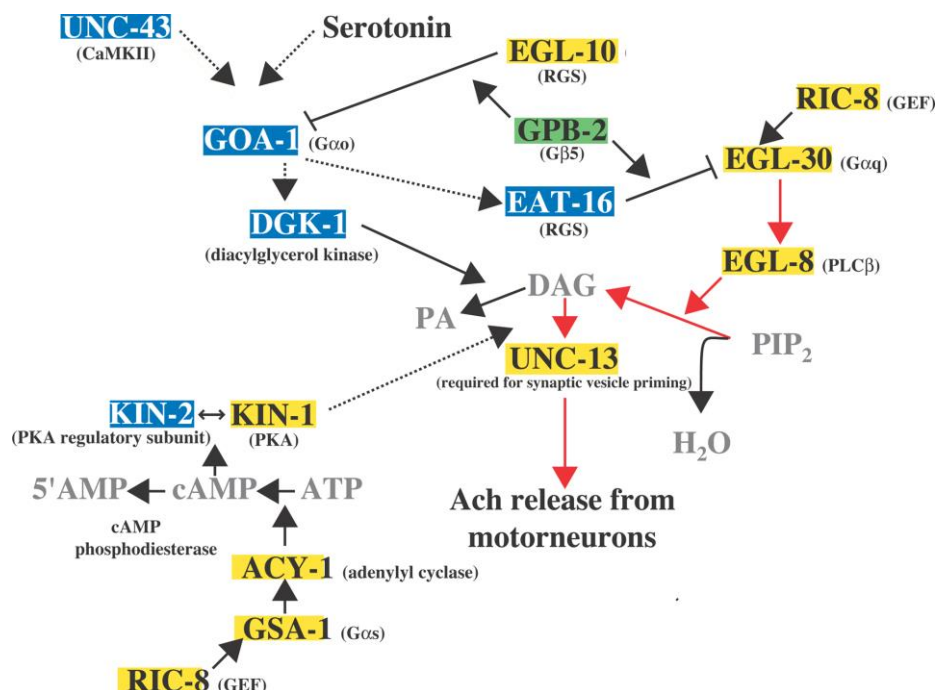


Figure 6. EGL-30 ($G\alpha_q$) and GOA-1 ($G\alpha_o$) signalling network. The graph was taken from WormBook, 2012. <http://www.wormbook.org> [58].

Besides serotonin, there are a few more neurotransmitters known acting upstream of GOA-1 receptors that are supposed to influence locomotion and regulate adaptive locomotion responses like FRMFamides, octopamine and dopamine [58, 65-67].

C. elegans shows adaptive locomotion behaviour in response to environmental cues like gustatory, olfactory and thermosensory stimuli. Swimming nematodes lowered their turning rate in liquids flowed through with an attractive odorant [68]. Swimming *C. elegans* responded to temperature variations with changes in their reorientation events [69]. Crawling *C. elegans* exhibit food choice behaviours like seeking for high quality food. The nematodes also exhibit locomotion strategies of roaming and dwelling depending on the food source [70, 71]. Well fed worms slow their speed of crawling when entering a food source. This locomotion behaviour was defined as "basal slowing response". Whereas short term starvation of worms lead to an "enhanced slowing response" because worms stop their movement almost totally when entering a bacteria lawn to gorge [67].

2. Materials and Methods

2.1. Maintenance and *C. elegans* strains

The nematodes were maintained at 20°C on NGM-agar plates with OP50 *E.coli* as food source following standard protocols [2]. Strains obtained from Caenorhabditis Genetics Center, USA: Bristol N2 used as wild type, *aak-2 (rr48)X*, *aak-2 (ok524)X*, *acr-2 (ok1887)X*, *akt-1 (mg306)V*, *cat-2 (e1112)II*, *che-2 (e1033)X*, *col-179 (ok3010)X*, *col-61 (ok2582)I*, *col-99 (ok1204)IV*, *daf-7 (e1372)III*, *dgk-1 (nu62)X*, *dpy-5 (e61)*, *eat-16 (sa609)I*, *eat-2 (ad465)II*, *egl-30 (ad805)I*, *egl-4 (n478)IV*, *egl-8 (e2917)V*, *egl-8 (md1971)V*, *far-4 (ok317)V*, *flp-18 (gk3063)X*, *gbp-2 (ad541)I*, *gbp-2 (pk751)I*, *goa-1 (n1134)I*, *goa-1 (syls9)*, *lon-2 (e678)X*, *mec-4 (e1611)X*, *odr-1 (n1936)X*, *pept-1 (lg1601)X*, *raga-1 (ok386)II*, *rict-1 (ft7)II*, *rict-1 (mg360)II*, *rsks-1 (ok1255)III*, *ser-2 (pk1357)X*, *ser-3 (ad1774)I*, *sgk-1 (ft15)X*, *snf-1(ok790)I*, *srp-3 (ok1433)V*, *tph-1 (mg280)II*, *trp-4 (sy695)I*, *unc-63 (x37)I*, *Y57A10C.6 (daf-22) (ok693)II*, *Y58G8A.4 (npr-5) (ok1583)V*. Strains *mgl-1 (tm1811)*, *mgl-2 (tm355)* and *npr-4 (tm1872)* were obtained from National Bioresource Project, Japan. The pGK10 strain was provided by Prof. Schnabel, TU Braunschweig, Germany. For further description see supplementary information 5. (**table S15**).

2.2. Chemicals, recipes and preparation of viscous liquids

NGM-agar plates, M9 buffer, PBS buffer and other media were prepared following standard protocols. The chemicals for maintaining, buffer preparation and supplementation experiments were obtained from Sigma-Aldrich, Germany or Carl Roth, Germany, unless otherwise mentioned. The viscous liquids were prepared of Carboxymethylcellulose (CMC) diluted in M9 buffer as described [72, 73], CMC was fully dissolved after heating by autoclaving. Viscosity of the CMC solutions was measured by Dr. Katrin Schrader with a rotation viscosimeter (MC 200, Fa. Paar, Austria) at Max Rubner-Institut, Kiel, Germany. The viscosity is declared in mPa s at a shear rate of 10 s^{-1} , details are shown in supplementary section 5. (**table S14**).

2.3. Food restriction regimes and amino acid supplementation

The dietary restriction (DR) protocol was recently developed in our laboratory [74]. In brief: *Ad libitum* (AL) fed control animals were grown on 5 g/l bactopectone (BD Biosciences, Germany) NGM-agar plates (9 cm in diameter) that were spread with a lawn of 3.75×10^8 bacteria cells (250 μ l of OP50 *E.coli* bacteria at $OD_{600nm} = 1.5$). To create the DR regime also 3.75×10^8 cells were seeded on NGM-agar plates lacking bactopectone. AL and DR plates were incubated at 37°C for about 16 h. The resulting bacteria quantity of AL and DR plates was approximately 2.5×10^{10} and 5×10^8 bacteria cells per plate, respectively. For the starvation regime (Starv), nematodes were put on 0 g/l peptone NGM-agar plates lacking bacteria for indicated hours. At the beginning of each experiment, nematodes were synchronized using 5 % sodium hypochlorite to receive the eggs. Usually 600 eggs were put on AL and DR plates, respectively. To guarantee constant food conditions the animals were transferred to freshly prepared plates after 54-58 h (\approx at L4 stage) of synchronization. For supplementation experiments, amino acids (Sigma-Aldrich, Germany) were dissolved in water and spotted on top of the prepared NGM-agar plates containing bacteria. To dissolve leucine, methionine and valine, the solutions were acidified by HCl (max. 10 % of total volume). The final amino acid concentration per plate was 0.02 - 4 mM. Plates humidified with water or water/ HCl, respectively, served as control.

2.4. Body bending analysis

To measure the number of body bends 3-10 nematodes were picked in a 10-30 μ l droplet of M9 buffer (or any other liquid) onto a glass slide. Afterwards, worms were immediately filmed with a Canon Legria HF20E camera (Canon, Germany) set on top of a Zeiss Stereo Discovery V8 microscope (Carl Zeiss AG, Germany). For body bending analysis on NGM-agar plates the worms were picked on 5 g/l peptone plates either with a bacterial lawn (AL control plates) or 5 g/l peptone NGM-agar plates lacking bacteria. The films of crawling worms were recorded immediately for 30 seconds with a Tucsen (S/N K3000176, 3.0 MD, Xintu Photonics Co., China) camera on top of a Zeiss Stemi 2000-C microscope (Carl Zeiss AG, Germany). The body bends of swimming worms were counted for 30 seconds, whereas body bends of crawling worms were counted as long as the worms crawled uniformly. Windows

media player classic (version 6.4.9.1) was used to play the films. One body bend describes the movement of the head region, which thrashes from one side to the other and back to the starting position. Only full thrashes were counted. Animals that often curled their bodies, crawled or swam backwards or stopped movement were discarded.

2.5. Quantification of speed of movement

The crawling and swimming speed of worms was analysed using the Multi-Worm Tracker (MWT) system described and provided by Swierczek et al. [75]. Films were recorded as described above and were converted from MTS format to AVI format and trimmed if necessary by using Aiseesoft MTS Konverter Software, version 6.2.16. The films were cropped to 1 cm for size adjustment and to calculate the exact pixel size per mm afterwards. Usually the films were recorded with a video resolution of 640x480 pixel for swimming and 1024x768 pixel for crawling worms. The films were taken with 25 or 30 frames per second (fps). The speed of the worms was analyzed over a time period of 10 seconds constant swimming or crawling. Mean values of speed (in MWT programme termed: s) were calculated from every single data point within the analyzed 10 seconds per worm.

The slip ratio was calculated following the assumptions of Gray and Lissmann, using the following equation: $100 [1-(V_x/V_w)]$. V_x describes the speed of progression, measured by MWT system, in mm/s. V_w is the speed of waves, defined by body bending frequency (f) per second and the length of the worms (λ), also analysed by MWT system: $V_w=f\lambda$ [53].

2.6. Flow cytometry and biochemical assays

The COPAS (Complex Object Parametric Analyzer and Sorter) Biosort system from Union Biometrica, Belgium, was used to sort the worms as well as to determine their time of flight (TOF) and extinction (Ext) values. As described by our laboratory, TOF and Ext values are proxies for the length and volume of the nematodes [76-78]. Standard instrument settings and thresholds were used as described, whereas sorting and gating regions were adapted for the particular purpose. To determine the amount of proteins usually 1000 adult worms were collected with the COPAS Biosort in M9 buffer which was replaced after sorting by 100 μ l of an modified NET-buffer

(50 mM Tris pH 7.5, 150 mM NaCl, 1 mM EDTA pH 8.0, 0.5 % CHAPS, 1x protease inhibitor cocktail (Roche Diagnostics GmbH, Germany)). A few 1.4 mm ceramic beads were added. Afterwards, worms were homogenized with the bead-beating homogenizer Precellys®24 (Peqlab, Germany) at 6500 rpm for 20 sec at 3 cycles, transferred into fresh tubes and centrifuged for 20 min at 4°C and 21,000 g. The resulting supernatant containing worm extract was stored at -20°C until analysis.

The amount of proteins was determined with the Pierce bicinchoninic acid (BCA) Protein Assay Kit (Thermo Fisher Scientific Inc., USA). The measurement was done using the Spectramax 190 (Molecular Devices, USA) at the wavelength of 562 nm. As an alternative method, protein content of the worms was determined by fluorometric measurement using the micro plate reader Infinite M200 (Tecan, Switzerland; excitation: 280 nm, emission: 330 nm) [79]. Dilutions of 2 mg/ml Albumin (BSA) stock were used as standards in both methods.

2.7. Amino acid analysis

For the AL fed control group 3000 worms were collected with the COPAS Biosort system. For the DR and Starv group, 8000 worms were collected. Worm extracts were prepared as described above with the exception that the extracts have not been centrifuged after homogenization in 500-600 µl NET-buffer. The samples were stored at -80°C until analysis. The samples were analyzed by Genaxxon BioScience GmbH (Ulm, Germany) to determine the total amino acid content (free plus protein bound). The amino acid analysis was performed using a standard HPLC-based method. In brief: 250 µl of worm extract was treated with HCl over night to hydrolyse peptides and proteins. To quantify free amino acids 200 µl of the sample were precipitated by 10 % sulfosalicylic acid and the supernatant was taken for amino acid analysis with the amino acid analyser LC3000 (Eppendorf-Biotronik, Germany). A calibration was performed using amino acid standards. The results were first adjusted to the worm number per sample to get the values per worm and were then normalized to total amino acid values to get the percentage of each amino acid per worm. The total amount of each amino acids analyzed by total hydrolysis minus the analyzed amount of free amino acids declares the amount of protein bound amino acids.

2.8. Statistical analysis

The illustrated data are expressed as mean values with standard deviation (SD \pm). A part of the statistical analyses were performed by Student's t-test and one-way analysis of variance (ANOVA) with Bonferroni multiple comparison test using GraphPad Prism 4.0 software, GraphPadSoftware, Inc., USA.

For specific questions a statistical consultation of Dr. Mario Hasler, Variationstatistik CAU Kiel, was accessed. A statistic mixed model was prepared to compare results more accurately over a wide range of experiments [80, 81]. For each experiment the mean values of body bends per seconds in Hertz (Hz) or speed in millimeter per second (mm/s) of AL fed N2 worms were calculated and set to 100 %. Then every single result within the same experiment was normalized to the calculated value. A two-way ANOVA (with interaction) was performed afterwards. For significance test simultaneous confidence intervals with coverage probability of 95 % ($1-\alpha$) were calculated. An equivalence test was used to determine which mutant strains did not response to dietary restriction compared to AL fed control. Therefore simultaneous confidence intervals with (simultaneous) coverage probability of 90 % ($1-2\alpha$) according the "two one-sided tests" (TOST) concept were calculated. All data were processed using R, version 2.15.1 obtained from The R Foundation for Statistical Computing. Resulting tables of the calculated confidence intervals concerning different questions are shown in supplementary section 5. (**table S2-S7**).

3. Results

3.1. Activity of the swimming gait but not crawling gait increases in response to different paradigms of dietary restriction

We investigated if dietary restriction (DR) alters *C. elegans* locomotor rate. For this purpose, N2 wild type hermaphrodites were exposed to DR during development. The locomotor activity of the swimming gait was analysed by picking synchronized adult AL or DR fed worms in liquid medium. Immediately, the frequency of body bends was counted. Well fed worms swam with a body bending (bb) frequency of 1.78 ± 0.03 (mean \pm Standard Deviation, SD) bb/s (hereinafter referred to as Hertz, Hz). Unexpectedly, DR accelerates body bending swimming frequency (BBSF, 2.39 ± 0.11 Hz) by about 50 % compared to control condition (**figure 7A**). To examine if this DR response is influenced by the presence of food, BBSF of worms was counted in liquid medium containing bacteria. AL fed adult worms swam in bacteria suspension with a BBSF of 1.73 ± 0.10 Hz, whereas BBSF of dietary restricted animals is markedly increased (2.41 ± 0.05 Hz) (**figure 7A**). Variation of temperature (15°C to 30°C) or osmolarity (0 to 300 mosmol/l) of the assay medium does not affect BBSF under AL and DR condition. Thus, DR induced acceleration of BBSF is a robust phenotype not affected by bacterial food, temperature or osmolarity (**figures S1A, S1B**).

Furthermore, we wanted to find out if dietary restricted worms translate the higher swimming frequency into higher speed of swimming. The speed was determined by using the Multi-Worm Tracker image-analysis software [75]. The analysis of swimming speed revealed a velocity of 0.33 ± 0.04 mm/s for AL fed worms and 0.39 ± 0.07 mm/s for dietary restricted worms, respectively (**figure 7B**). The presence of bacterial food in the assay medium did not affect speed of swimming of AL and DR fed worms. Additionally, the economy of swimming was determined by calculating the slip ratio between speed of waves and speed of progression [53] (**table 1**). The efficiency of forward swimming was found to be slightly more effective in dietary restricted wild type worms (73.8 %) compared to AL fed worms (79.4 %). Thus, DR accelerates BBSF as well as speed of swimming in the presence and absence of food. Next we investigated if DR also increases the activity of the crawling

gait. Well fed worms, picked on assay plates without bacteria, crawled with a body bending frequency of 0.68 ± 0.04 Hz and a speed of 0.30 ± 0.06 mm/s. Dietary restricted worms crawled with slightly lower body bending frequency (0.64 ± 0.04 Hz) and speed (0.19 ± 0.05 mm/s) (**figures 7A, 7B**). The body bending crawling frequency (BBCF, 0.58 ± 0.02 Hz) and speed (0.24 ± 0.05 mm/s) of AL fed worms were decreased when the worms were transferred to plates containing bacteria compared to those transferred to plates lacking bacteria (**figures 7A, 7B**). This effect, known as “basal slowing response”, is even more pronounced in dietary restricted worms (“enhanced slowing response”) [67]. In summary, DR accelerates BBSF and speed of swimming, whereas BBCF and speed of crawling is decreased in response to DR. The activity of the swimming gait is not influenced by bacterial food, whereas the activity of the crawling gait is reduced in the presence of bacteria.

Next, we determined the BBSF in different life stages of wild type worms. Compared to AL fed worms, dietary restricted L4 larvae, young adults as well as older reproductive adults show 31 % to 69 % higher BBSF (**figure 7C**). To investigate the persistence of the DR response, dietary restricted young adult worms were exposed to AL condition for further 24 h to 96 h. At every tested time point, BBSF of re-fed worms was between AL and DR fed worms (**figure 7C**). Accordingly, the DR induced higher BBSF was not completely abolished after re-feeding. To determine if the accelerated BBSF is also inducible by other DR regimes, starvation and genetic models of DR were used as alternative paradigms of dietary restriction. First the starvation paradigm was examined. Therefore, AL fed L4 larvae and adult worms were starved for several hours. In both developmental stages, we observed an almost linear increase in BBSF during the first 8 h of starvation. A further 8 h starvation period did not lead to an acceleration of BBSF as observed after first 8 h of starvation. In conclusion, starvation of AL fed larvae and adults induces a profound acceleration of BBSF (**figure 7D**). Moreover, this starvation response was 8.4 % higher compared to the dietary restriction response (**figure 7E**).

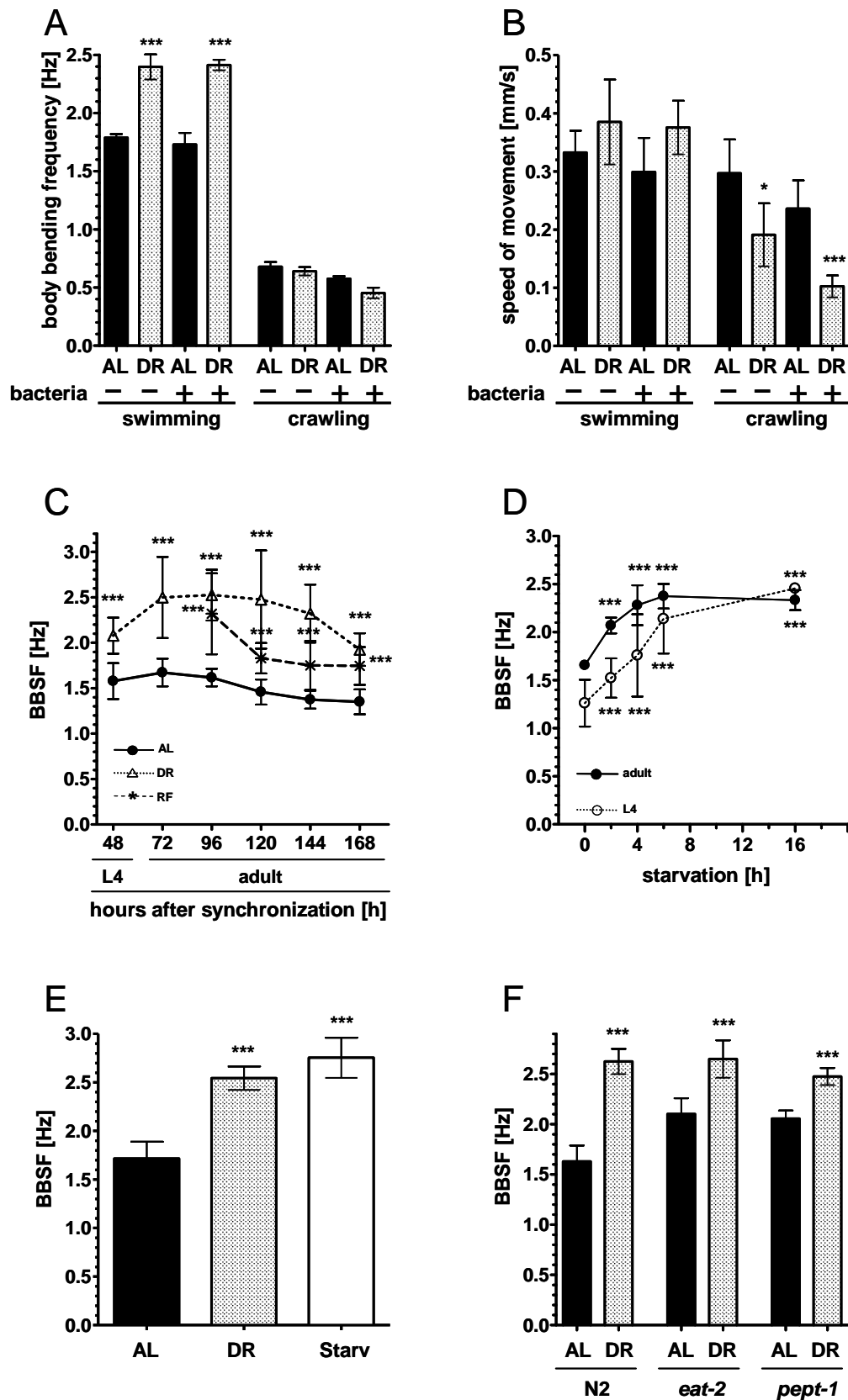


Figure 7. Influence of dietary restriction regimes on locomotory activity of *C. elegans*. **A)** Body bending crawling frequency (BBCF) and body bending swimming frequency (BBSF), **B)** crawling and swimming speed (mm/s) of *ad libitum* and dietary restricted fed adult (72 hours after synchronization) wild type worms. *Ad libitum* fed (AL, black) and dietary restricted

(DR, grey) wild type worms, either crawling on 5 g peptone NGM-agar plates with (+) or without (-) a bacterial lawn. BBCF and BBSF are expressed as body bends per second in Hertz (Hz). Swimming assay was performed in M9 buffer (-) or bacteria containing suspension ($OD_{600} = 1.5$) (+). Mean values (\pm SD) of 8 (N) experiments for BBCF with 4-7 (n) worms tested in each experiment and condition; crawling velocity N = 7 with n = 5-6. N = 5 with n = 3-6 for BBSF and N = 6 with n = 3-6 for swimming velocity. AL group was statistically compared to DR of the same condition.

C) BBSF of synchronized wild type L4 larvae (48 h) and reproductive adults (72-168 h) under AL (black circles), DR (white triangles, dotted line) and re-feeding (RF, asteriks, dashed line) conditions. AL and DR regime was performed from egg (0 h) to reproductive stage (168 h). For re-feeding condition worms were kept under DR regime for 72 h and afterwards put on AL condition. Analysis of BBSF was carried out every 24 h. N = 3; n = 18 worms tested per condition. Results of AL feeding condition were compared to DR and RF of the same age.

D) Acceleration of BBSF due to starvation. AL fed L4 (white circles, dotted line) and adult (black circles) wild type worms were starved for 2-16 h. Afterwards, BBSF was analysed. N = 2 experiments and n = 12 worms per condition. AL was compared to starved group of the same time point.

E) BBSF of synchronized adult wild type worms exposed to AL (black), DR (grey) or starvation (Starv, white). For starvation condition, AL fed L4 larvae were put on bacteria-free plates for 18-24 h. N = 49 experiments for AL and DR; n = 297 worms tested for AL; DR n = 283; Starv N = 13, n = 90.

F) BBSF of *eat-2* and *pept-1* mutants, genetic models for DR and protein restriction, respectively. Wild type worms were analysed 72 h after synchronization (adult stage), N = 3, n = 27; *eat-2* after 96 h (adult), N = 3 n = 21; *pept-1* after 80-90 h (adult), N = 2 n = 12. Comparisons: AL vs. DR condition of the same strain as illustrated; AL fed wild type vs. AL fed mutant strains, not illustrated, but also significant in every case (**p < 0.001).

A)-F) analysed by one-way ANOVA and Bonferroni multiple comparison as post test (*p < 0.05; **p < 0.001).

A), B), E), F) statistical results were confirmed by mixed model analysis of two-way ANOVA and simultaneous confidence intervals with 95 % coverage probability, for details see supplementary data (tables S3, S5, S6).

To approve that DR accelerates BBSF, we used the *eat-2* (*ad465*) mutant strain. In *eat-2* mutant worms the pharyngeal pumping rate and ingestion of food is reduced and therefore this strain serves as a genetic model for DR [82, 83]. Compared to N2 wild type worms, adult *eat-2* mutant worms exhibit 28 % higher BBSF (2.10 ± 0.15 Hz). Dietary restriction further increases BBSF of *eat-2* worms. Interestingly, BBSF of DR fed wild type and *eat-2* mutant worms are very similar (2.62 ± 0.13 Hz versus 2.65 ± 0.16 Hz), suggesting an upper limit of BBSF (**figure 7F.**) Finally, *pept-1* (*lg1601*), a genetic model of protein restriction, was analysed [30]. BBSF of AL fed adult *pept-1* mutant worms was 26 % higher in comparison to AL fed adult wild type worms (**figure 7F**). RNAi of *pept-1* (K04E7.2) showed similar results (data not shown). As observed in *eat-2*, DR increases the BBSF of *pept-1* worms to frequencies found in dietary restricted wild type worms. Taken together, BBSF of adult worms increases in response to different paradigms of dietary restriction.

3.2.1. DR fed worms have lower swimming power per body bend and a reduced protein content, indicating less muscle mass than AL fed worms

Supposing that dietary restricted *C. elegans* consume less energy, it is remarkable that those worms swim with an accelerated BBSF and speed. These data raised the question concerning the muscular strength of DR worms. Expecting, that worms swimming in higher viscous liquids have to extend their swimming power [72, 73], the BBSF and speed of AL fed and DR wild type worms in viscous liquids was compared. We observed a decrease of BBSF and speed with increasing viscosity (**figures 8A, 8B**). The enhanced BBSF in response to DR was also detectable in the viscous liquids. Calculating the decline of BBSF in the liquid media between 1 and 289 mPa s, it turned out, that AL fed worms decrease their BBSF by about 17.8 % (1.68 Hz at 1 mPa s to 1.38 Hz at 289 mPa s), whereas dietary restricted worms show a decline of 31.2 % (2.4 Hz at 1 mPa s to 1.65 Hz at 289 mPa s). Similar results were found for speed of swimming. AL fed worms decrease their velocity by 25.7 % (0.29 mm/s at 1 mPa s to 0.22 mm/s at 289 mPa s), whereas velocity of DR fed worms was reduced by 44.7 % (0.37 mm/s at 1 mPa s to 0.2 mm/s at 289 mPa s). Taken together, dietary restricted worms showed lower swimming power than AL fed wild type worms.

Table 1. Overview of parameters determining slip ratio of different strains at AL and DR.

Slip ratio in percent was calculated as described in 2.5. Body length and speed of swimming were analysed with Multi-Worm Tracker system.

Strain	Feeding condition	Body length [mm] ± SD	BBSF [Hz] ± SD	Speed of waves (V _w)	Speed of swimming (V _x) [mm/s]	Speed per body length	V _x / V _w	Percent slip [%]
<i>N2</i>	AL	0.90 ± 0.04	1.79 ± 0.03	1.62	0.33 ± 0.04	0.367	0.21	79.41
<i>N2</i>	DR	0.61 ± 0.04	2.40 ± 0.10	1.47	0.39 ± 0.07	0.629	0.26	73.81
<i>dgk-1</i>	AL	0.81 ± 0.05	1.82 ± 0.04	1.47	0.32 ± 0.06	0.392	0.22	78.47
<i>dgk-1</i>	DR	0.67 ± 0.04	1.99 ± 0.07	1.34	0.31 ± 0.03	0.466	0.23	76.57
<i>egl-8 (md1971)</i>	AL	0.78 ± 0.07	1.63 ± 0.10	1.27	0.23 ± 0.01	0.288	0.18	82.30
<i>egl-8 (md1971)</i>	DR	0.51 ± 0.04	1.95 ± 0.12	0.99	0.21 ± 0.03	0.408	0.21	79.08
<i>gbp-2 (ad541)</i>	AL	0.81 ± 0.05	2.04 ± 0.07	1.66	0.39 ± 0.05	0.479	0.23	76.50
<i>gbp-2 (ad541)</i>	DR	0.65 ± 0.02	2.17 ± 0.09	1.41	0.37 ± 0.13	0.567	0.26	73.85
<i>ser-3</i>	AL	0.78 ± 0.06	2.05 ± 0.11	1.60	0.39 ± 0.03	0.505	0.25	75.41
<i>ser-3</i>	DR	0.60 ± 0.03	2.19 ± 0.10	1.32	0.37 ± 0.09	0.620	0.28	71.72

The skeletal muscle accounts for approximately 40 % of mammalian body mass and serves as a predominant protein resource [84, 85]. Thus, supposing an equal ratio in

C. elegans, the protein content of a worm might indicate the amount of muscle mass. Regarding this idea, the protein content of dietary restricted and AL fed control worms was determined using three different methods. Compared to AL condition, dietary restricted worms contain about 60 % lower protein content (**figures 8C, S2A, S2B**). To further compare the body size of AL fed and dietary restricted wild type worms, body length and body volume was analyzed in a large number of worms using a flow cytometry-based method [76]. We found a reduced body length (66 % of AL fed worms) and body volume (54 %) in DR fed worms (**figures S2C, S2D**). Thus, the decreased protein content, assuming a reduced muscle mass, of DR fed worms corresponds to their reduced body size. To get a better impression of the speed of DR fed worms, the speed was normalized to the actual body size of the worms (**table 1**). This allometric ratio revealed that the smaller dietary restricted wild type worms swam even about 2-fold faster than AL fed worms. Together, DR fed worms might compensate their less muscle mass by increasing body bending frequency to maintain or even increase speed of swimming.

3.2.2. Body size, body elasticity and basal BBSF under *ad libitum* condition is not relevant to explain the higher BBSF in response to dietary restriction

Since worms exposed to DR are smaller than AL fed worms, we examined if the body size affects BBSF, using mutant strains with altered body size. *Che-2* (*e1033*) mutant worms are phenotypic small, whereas *egl-4* (*n478*) mutant worms are larger than N2 wild type worms. The BBSF of these mutant strains was similar to wild type under AL condition (1.77 Hz N2, 1.84 Hz *che-2*, 1.77 Hz *egl-4*). The large *egl-4* mutant worms showed a similar BBSF response to DR (2.55 Hz) than the wild type, whereas the response of the small *che-2* is reduced by 41 % (**figure 8D**). Thus, an inverted relationship between body size and BBSF is not obvious.

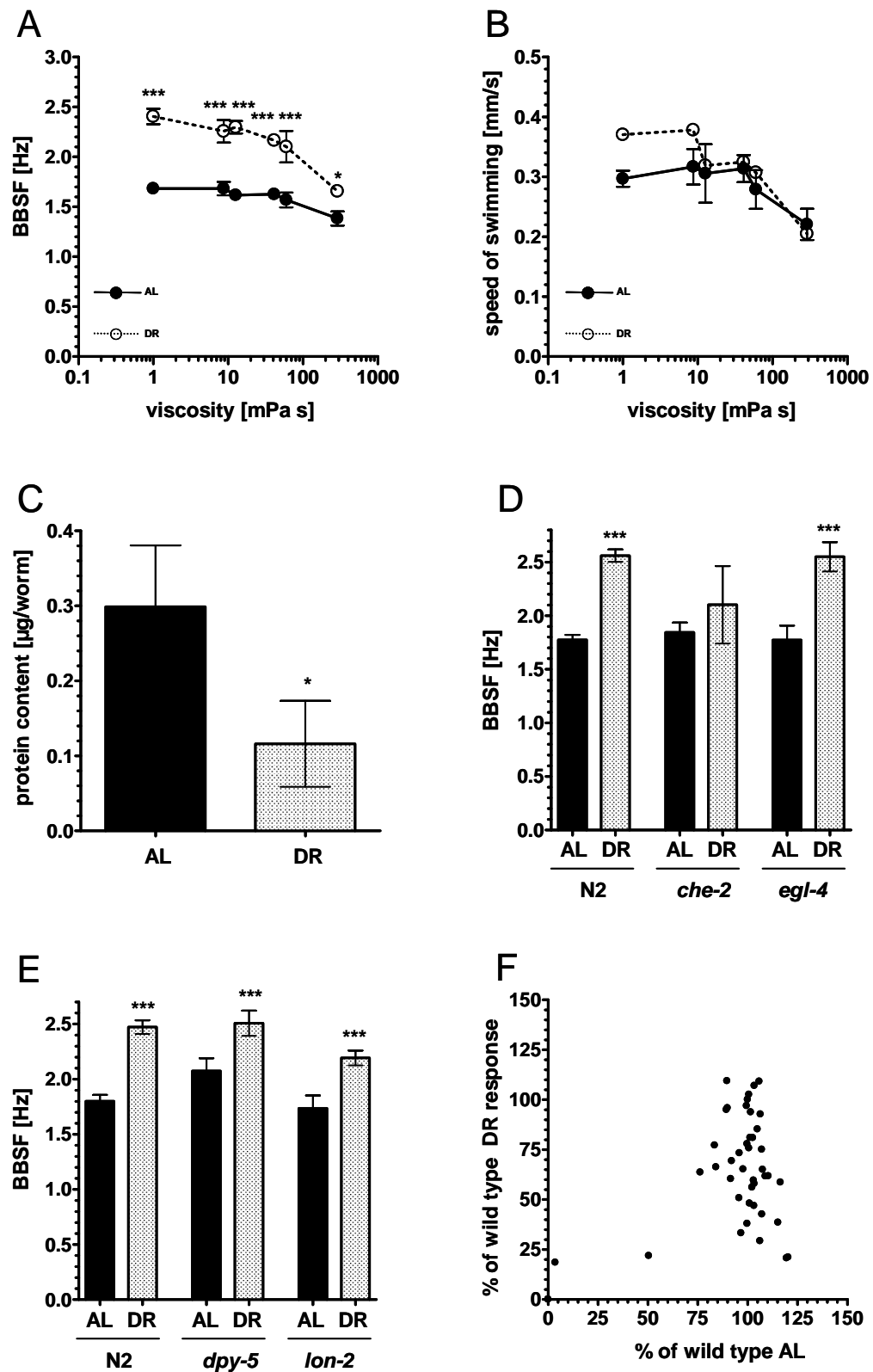


Figure 8. Influence of DR on swimming activity of adult wild type worms in assay media with altered viscosity and adult mutants with altered body size and stiffness. Influence of viscosity of the assay medium on BBSF (A) and swimming velocity (B) of N2. Comparison of BBSF and velocity of AL fed (AL, black circles, straight line) and dietary restricted (DR, white circles, dotted line) adult wild type worms swimming in M9 buffer

(1 mPa s) and viscous liquids of CMC buffer. BBSF is expressed as body bends per second (BBSF, in Hz). Swimming velocity (mm/s) was analysed by multi-worm tracker. Mean values (\pm SD) of N = 3 experiments and n = 15 worms per condition were calculated.

C) Protein content of AL fed (AL, black) and dietary restricted (DR, grey) adult wild type worms. The protein content is expressed in microgram (μ g) protein per worm and was analysed by bicinchoninic acid (BCA) protein assay. Means (\pm SD) of N = 3 experiments are shown.

BBSF of AL (black) and DR (grey) fed mutants, respectively, with altered body size (**D**) and elasticity (**E**). Values are illustrated as means (\pm SD) of N = 1-5 with n = 4-6.

F) Correlation of BBSF of AL fed and dietary restricted mutant worms, normalized to wild type AL BBSF and wild type DR response, respectively. Correlation results in $R^2 = 0.2156$.

A)-E) One-way ANOVA and Bonferroni multiple comparison as post test was performed, comparing AL and DR group ($p > 0.05$, * $p < 0.05$; *** $p < 0.001$).

Based on biomechanical analysis, it has been shown, that muscle power during swimming is primarily devoted to the elasticity of the worm's body [86]. The cuticle pro-collagen mutant *dpy-5* (*e61*) was found to be more softer than N2, whereas the long and skinny *lon-2* (*e678*) mutant worms were found to be stiffer than the wild type worms [86]. These mutants were used to examine if the worms body stiffness may influence BBSF. Under AL feeding condition *lon-2* and *dpy-5* mutant worms showed similar or slightly increased BBSF, compared to wild type (1.8 Hz N2, 2.1 Hz *dpy-5*, 1.87 Hz *lon-2*). In both mutants, the BBSF response to DR is 35 % reduced compared to wild type response to DR (**figure 8E**). Since a microarray analysis of genes regulated in response to DR (unpublished data, Palgunow, Klapper, Döring) showed a down-regulation of collagen genes, we further examined if a defective collagen structure affects body bending and BBSF using *col-61* (*ok2582*), *col-99* (*ok1204*) and *col-179* (*ok2010*) mutant strains. *Col-179* mutant worms showed an increased BBSF after AL feeding and a reduced response to DR, whereas *col-61* and *col-99* mutant worms showed body bending swimming frequencies similar to wild type (for details see **table S1**). Taken together, body elasticity of the worms and selected collagen genes have no substantial influence on BBSF under AL condition and DR induced acceleration of BBSF. Because BBSF under AL condition of above tested mutant strains differ from each other, we asked, if the BBSF response to DR correlates to BBSF under AL condition. For this purpose, we compared BBSF data obtained from several mutant strains, being analysed in this work in more detail. We found no significant correlation ($R^2 = 0.22$) between BBSF obtained under AL and DR condition (**figure 8F**).

3.3. Dietary restriction induced acceleration of body bending swimming frequency is partially mediated by pathways sensing free amino acids (TOR) and cellular energy status (AMPK)

Because AL fed *pept-1* mutant worms mimicking a protein restriction, show higher BBSF, we supposed that amino acid metabolism contributes to the DR induced swimming phenotype. Initially, we performed an amino acid analysis of wild type worms undergoing AL feeding or DR. As expected, the pattern of protein and peptide bound amino acids are very similar under AL and DR condition (**figure S3A, B**). In contrast, analysis of free amino acids showed partly distinct variations related to DR (**figure 9A, B**). The relative concentrations of the branched-chain amino acids (BCAA) leucine, isoleucine and valine are approximately 64 % lower under DR condition compared to AL feeding. Considerable reductions were also found for lysine (31%), tyrosine (71 %), methionine (64 %) and phenylalanine (47 %). Whereas the relative concentrations of glutamic acid (78 %), alanine (62 %), aspartic acid (120 %) and glycine (118 %) are increased due to DR. Because of the observed, reduced levels of certain amino acids, the BBSF of DR fed worms supplemented with certain amino acids was determined. None of the supplemented amino acids were able to prevent DR induced acceleration of BBSF completely (**figure S3C**). Taken together, DR alters the pattern of free amino acids and these changes may partially contribute to the DR response.

It has been postulated, that loss of function of the metabotropic G-protein coupled glutamate receptor MGL-2 mediates a systemic starvation response via sensing of free amino acids [38]. Therefore, mutants of MGL-2 and its paralog MGL-1 were examined. Compared to wild type, *mgl-2* (*tm355*) and *mgl-1* (*tm1811*) mutant adults increase their BBSF in response to DR to 72 % and 63 %, respectively (**figure 9C**). Because TOR signalling is of central importance for maintaining cell functions as well as sensing of growth factors, the intracellular energy status and nutrients like leucine, selected mutant strains of the TOR pathway were studied [31, 87]. This includes *rsks-1* (*ok1255*), *raga-1* (*ok386*), *rict-1* (*ft7*) and *rict-1* (*mg360*), *akt-1* (*mg306*) and *sgk-1* (*ft15*). RSKS-1 is the worm homologue of mammalian protein S6 kinase (S6k) and is required for protein synthesis [88]. *Raga-1* is the *C. elegans* ortholog of the ras-related GTPase RagA which functions upstream of TOR [89]. The ortholog gene of mammalian Rictor, a part of the TOR complex 2 (TORC2) is *rict-1*. Together with

TORC2 components AKT and SGK, Rictor regulates the fat content, growth and aging of the organism [90]. The DR induced BBSF response of *rsks-1*, *raga-1* and *rict-1 (ft7)* mutant worms are similar to the N2 wild type response. In contrast, the DR response of *rict-1 (mg360) sgk-1 (ft-15)* and *akt-1 (mg306)* is reduced to 42-61 % of wild type level (**figure 9C**). Another component of the TOR signalling network is the AMP-activated kinase AMPK. AMPK senses cellular energy status and its activity depends on the ATP/AMP ratio [91]. We tested two mutant alleles of *aak-2*, which functions as a catalytic subunit of heterotrimeric AMPK. The DR induced BBSF response of the *aak-2 (rr48)* mutant was reduced to 59 % of wild type increase. A second allele of *aak-2 (ok524)* showed also a reduced DR response (**figure 9C**). In summary, we hypothesize a relationship between pathways sensing (free) amino acids (TOR) and cellular energy status (AMPK) and DR induced acceleration of BBSF based on the minor response of those mutant strains to DR.

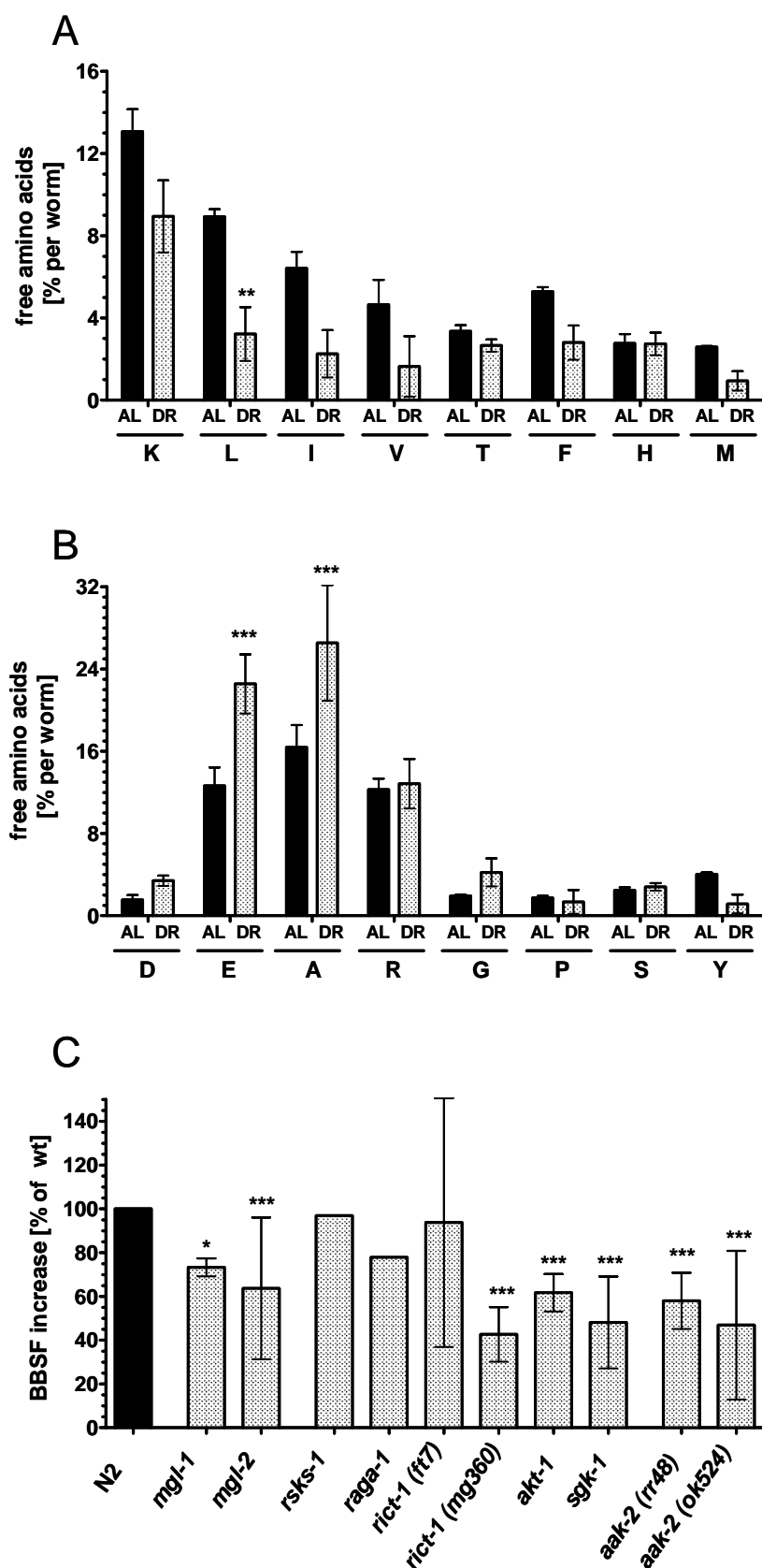


Figure 9. Free amino acid content of *ad libitum* fed and dietary restricted adult wild type worms as well as DR induced acceleration of BBSF of adult mutant worms deficient in glutamate, TOR or AMPK signalling.

Relative proportion of essential (A) and non-essential (B) free amino acids of AL fed (black) and dietary restricted (grey) adult wild type worm. The mean values (\pm SD) of N = 3

independent experiments with $n = 8654$ AL worms and $n = 23124$ DR worms in total are shown. Data of bound amino acids are shown in detail in figure S3A and S3B, details in tables S8-S13.

C) The relative BBSF increase of dietary restricted adult mutant worms deficient in glutamate (*mgl-1*, *mgl-2*), TOR (*rsk-1*, *raga-1*, *rict-1*, *akt-1*, *sgk-1*) or AMPK (*aak-2*) signalling pathway is illustrated. The BBSF increase of dietary restricted mutant worms (grey), compared to AL condition, was normalized to BBSF increase of wild type (black).

A)-C) Statistical analysis was performed using one-way ANOVA and Bonferroni multiple comparison as post test ($p > 0.05$; $**p < 0.01$; $***p < 0.001$). **C)** Further statistical analysis was performed by mixed model analysis of two-way ANOVA and simultaneous confidence intervals with 95 % coverage probability are shown in table S6.

3.4.1. Mutant strains of the neuropeptide FLP-18 and its G-protein coupled receptor NPR-5 slightly suppress the DR induced acceleration of BBSF

FMRamide related neuropeptides function as signal transmitter through neurons and play an important role in mediating olfactory, gustatory and thermosensory behaviours in *C. elegans*. FLP-18 is one of these peptides, expressed by the AIY-amphid interneuron, and involved in foraging. FLP-18 mediates its effect via the G-protein coupled neuropeptide receptors NPR-4 and NPR-5 [92-94]. To investigate if the DR induced higher BBSF is activated by an enhanced foraging on diminished food supply *flp-18* (*gk3063*), *npr-4* (*tm1782*) and *npr-5* (*ok1583*) deletion mutant worms were subjected to DR and their swimming frequency was analysed. The BBSF response to DR of *flp-18* and *npr-5* mutant worms was 66 % and 61 % of wild type, respectively. *Npr-4* mutant worms increase their BBSF in response to DR to the same level as N2 wild type (**figure 10A**). Thus, FLP-18 and NPR-5, but not NPR-4 are slightly involved in mediating the DR swimming response.

3.4.2. The G-protein coupled octopamine receptor SER-3 but not the tyramine receptor SER-2 is required for DR induced acceleration of BBSF

In order to examine if serotonin is involved in DR response, the serotonin lacking *tph-1* (*mg280*) strain was tested. *Tph-1* mutant worms showed an abnormal crawling phenotype and also its swimming behaviour was uncoordinated (**table S1**). Next, we examined, whether SER-3 plays a role for the increase of BBSF in response to DR, since the octopamine receptor SER-3 mediates serotonin dependent inhibition of locomotion [95]. We found that the DR response of the deletion mutant *ser-3* (*ad1774*) was reduced to 21 % of wild type level (**figure 10A**). With respect to speed

of swimming, the *ser-3* mutant showed no response to DR. The presence of bacterial food in the assay medium did not influence the minor DR responses of *ser-3* mutant worms (**figure 10B**). To investigate if tyramine, a precursor molecule for octopamine synthesis, affects the DR induced acceleration of BBSF, a deletion mutant of the tyramine receptor *ser-2* (*pk1357*) was analysed [96, 97]. *Ser-2* showed a similar response to DR like the wild type (**figure 10A**). The G-protein coupled octopamine receptor SER-3, but not the tyramine receptor SER-2, is required for DR induced acceleration of BBSF.

3.4.3. The synaptic $G\alpha_o$ - $G\alpha_q$ signalling network, which regulates acetylcholine release, is required for the DR induced acceleration of BBSF and speed of swimming

Muscle contraction is induced by acetylcholine (ACh) release from motor neurons. The signal transduction regulating ACh release from motor neurons is derived by interaction of the activating EGL-30 ($G\alpha_q$) and inhibiting GOA-1 ($G\alpha_o$) pathways [59]. Using deletion mutants, we explored if EGL-30 and its down-stream target EGL-8 are involved in the DR induced acceleration of BBSF. EGL-30 encodes a heterotrimeric G-protein α subunit, which functions as an upstream effector of EGL-8 [98]. *Egl-8* encodes a phospholipase $C\beta$, which is responsible for degradation of phosphatidylinositol (4,5)-bisphosphate to DAG and inositol (1,4,5)-triphosphate [59, 63].

Interestingly, the *egl-30* (*ad805*) mutant showed defective movement on plates and exhibits also an almost paralyzed swimming phenotype. For this reason, the *egl-30* mutant was excluded from further analysis. The BBSF response to DR of the *egl-8* (*e2917*) mutant was 60 % of wild type response. For confirmation, a second *egl-8* (*md1971*) mutant allele was analysed. The DR induced acceleration of BBSF of this *egl-8* mutant was only 30 % of wild type response swimming in liquid media without bacteria (**figure 10A**). In contrast, swimming in liquid media containing bacteria, the DR induced BBSF response of the *egl-8* (*md1971*) mutant was similar to wild type response, whereas AL *egl-8* (*md1971*) mutant worms, swimming in assay medium with bacteria, showed a reduced BBSF. Compared to wild type, the swimming speed of *egl-8* (*md1971*) was also reduced (**figures 10B, 10C**). Taken together, EGL-8, a component of the activating EGL-30 ($G\alpha_q$) pathway, might contribute to DR induced acceleration of BBSF.

The G-protein GOA-1 and its down-stream target DGK-1 are key components of the inhibiting $G\alpha_o$ signalling pathway. *Dgk-1* encodes a diacylglycerol kinase reducing the concentration of the second messenger diacylglycerol (DAG) [64]. Similar to the phenotype of the *egl-30* mutant, a gain-of-function *goa-1 (syls9)* mutant showed paralyzed swimming phenotype and abnormal crawling behaviour. Mutant worms with a loss-of-function mutation of *goa-1 (n1134)* showed a normal swimming phenotype under AL condition but their BBSF response to DR was markedly reduced. The DR response of *goa-1 (n1134)* was 37 % compared to the wild type response. In line with this, BBSF of *dgk-1 (nu62)* mutant worms showed only a minor response to DR (29 % of N2) (**figure 10A**). With respect to speed of swimming, *dgk-1* mutant worms showed no essentially response to DR. Similar to wild type, BBSF as well as speed of swimming of *dgk-1* mutant worms were unaffected by the presence of bacterial food (**figures 10B, 10C**). In summary, mutant strains of the inhibiting GOA-1 ($G\alpha_o$) signalling network showed a drastically reduced BBSF response to DR compared to wild type.

To further examine the role of the $G\alpha_o$ - $G\alpha_q$ signalling network on DR induced acceleration of BBSF, genes encoding proteins interacting with the EGL-30 ($G\alpha_q$) and GOA-1 ($G\alpha_o$) pathway, were analysed. Interacting proteins of this pathway are the Regulator of G-protein signalling (RGS) EAT-16 and the ortholog of $G\beta_5$, GBP-2 [62, 63]. The DR response of *eat-16 (sa609)* mutant worms was 69 % of wild type increase. Compared to wild type, DR response of *gbp-2 (pk751)* mutant worms result in 38 % increase of BBSF. Another deletion mutant of *gbp-2*, *gbp-2 (ad541)*, showed a DR response of only 20 % of wild type (**figure 10A**). Speed of swimming of *gbp-2 (ad541)* mutant worms is slightly reduced in response to DR. The presence of bacteria in assay medium did not influence BBSF and speed of swimming of *gbp-2 (ad541)* mutants (**figures 10B, 10C**).

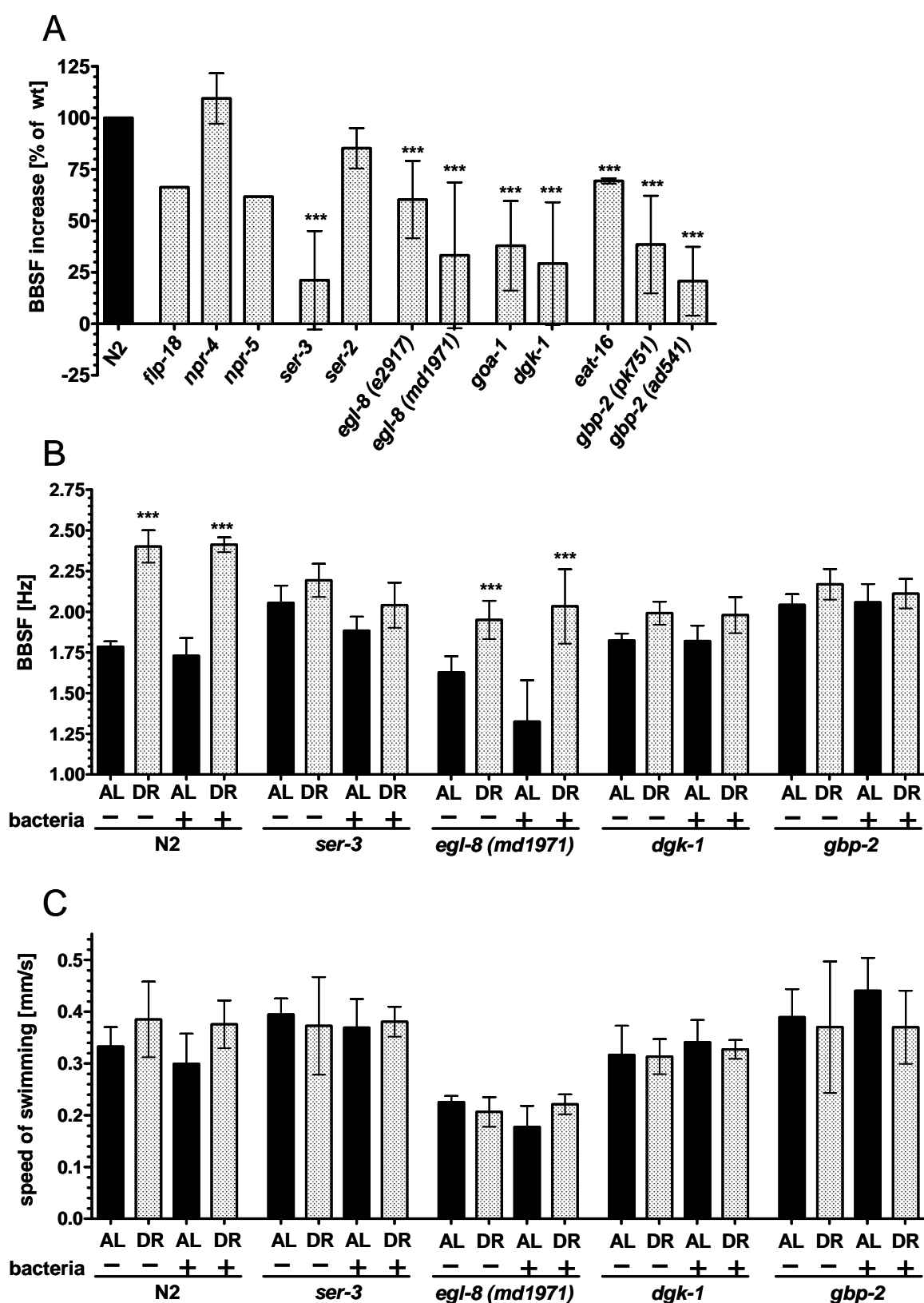


Figure 10. DR induced acceleration of BBSF and swimming speed in mutant strains of the EGL-30 and GOA-1 signalling network.

A) Relative BBSF increase of dietary restricted adult mutant worms deficient in FMRFamide neuropeptide (*flp-18*, *npr-4*, *npr-5*), tyramine and octopamine (*ser-2*, *ser-3*) or $G_{\alpha o}$ - $G_{\alpha q}$ (*egl-8*, *goa-1*, *dgk-1*, *eat-16*, *gbp-2*) signalling pathway are illustrated. The BBSF increase of dietary

restricted mutant worms (grey), compared to AL condition, was normalized to BBSF increase of wild type (black). Values are illustrated as means (\pm SD).

BBSF (**B**) and swimming speed (**C**) of selected mutant strains under *ad libitum* (AL) and dietary restricted (DR) feeding condition. Swimming activity was determined in the presence (+) or absence (-) of bacterial food. N= 2-5 with n = 3-6 analysed worms per condition and per experiment for BBSF analysis and N = 3-6 with n = 3-6 analysed worms per condition and per experiment for swimming speed. Statistical comparison illustrates the comparison of AL vs. DR mutant worms swimming in the same kind of liquid. Differences concerning the kind of liquid were only detected for AL fed *egl-8* mutant worms, which showed a significantly declined BBSF in bacteria containing suspension (**p < 0.01, not illustrated in graph B).

A)-C) Statistical analysis was performed by one-way ANOVA and Bonferroni multiple comparison as post test (p > 0.05, ***p < 0.001). Further statistical results concerning different questions were performed by mixed model analysis of two-way ANOVA. Simultaneous confidence intervals with 95 % coverage probability, are shown in table S6.

The crawling frequency and speed of crawling of *gbp-2* (*ad541*) in comparison to wild type was analysed, either on plates lacking or containing a bacteria lawn. In contrast to wild type worms, BBCF of *gbp-2* (*ad541*) mutant worms was higher in response to DR. The speed of crawling was similar to wild type at which the slowing response due to DR was reduced (**figures 11A, 11B**). Summing up, GBP-2, a central component of the $G\alpha o$ - $G\alpha q$ signalling network, shows a reduced swim response to DR, whereas its crawling response to DR is similar to wild type. These data indicate a strong relationship of GBP-2 to mediate the DR response at the swimming gait.

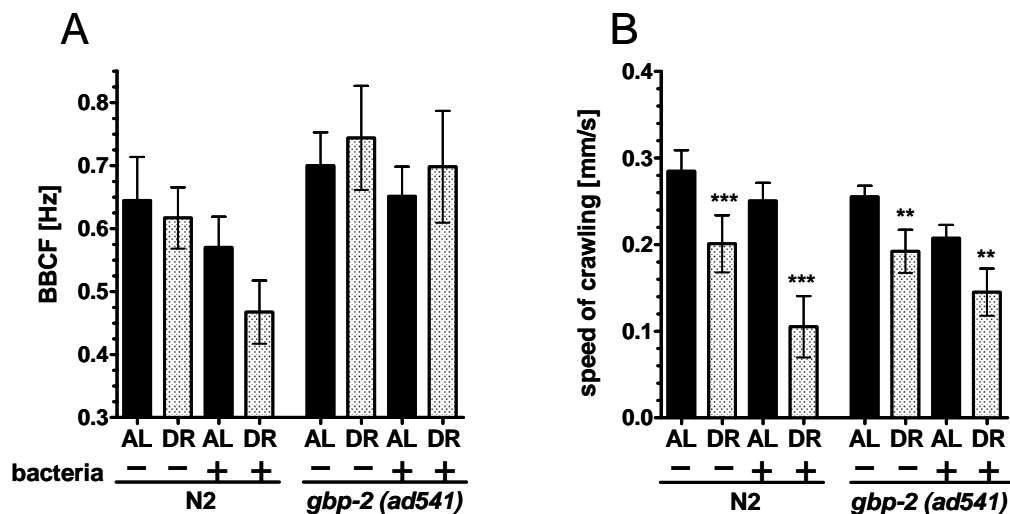


Figure 11. Influence of dietary restriction on body bending crawling frequency and crawling speed of adult *gbp-2* mutant deficient in EGL-30 and GOA-1 signalling.

Body bending crawling frequency (BBCF, **A**) and crawling speed (**B**) of *ad libitum* (AL) fed and dietary restricted (DR) adult wild type and *gbp-2* (*ad541*) worms. Crawling activities were determined on plates containing (+) or lacking (-) bacterial food. Data represents mean (\pm SD) values. N = 4 with n = 3-6 analysed worms per condition and per experiment. Statistical

comparison of AL vs. DR fed worms crawling under the same condition is illustrated and was performed by one-way ANOVA and Bonferroni multiple comparison as post test ($p > 0.05$, $**p < 0.01$, $***p < 0.001$). Differences concerning the influence of bacteria on plate resulted in no significant differences for *gbp-2 (ad541)*, but a significant difference between DR N2 worms crawling on plates with bacteria compared to crawling on empty plates was recorded ($***p < 0.001$, not illustrated). Further statistical analysis was performed following mixed model and two-way ANOVA as described in detail in tables S3 and S5.

4. Discussion

Animals need a well developed neuromuscular system for coordinated locomotion and to adapt to different environments. Alternative forms of locomotion, referred to as gaits, have been described across the species. Gait adaptation not only occurs in animals with a limb system but is also present in invertebrates. *Caenorhabditis elegans* (*C. elegans*) switches between a crawling gait while moving through dense surfaces and a swimming gait when entering liquids [55-57, 99]. The transition between gaits is mediated by different neural circuits [53, 99-102]. Dopaminergic neurons regulate the switch from swimming to crawling, whereas serotonergic neurons regulate the switch from crawling to swimming [56, 57, 101]. From a functional point of view, the gaits differ in their kinematics of the generated undulations and their pattern of muscle activity [56]. In terms of locomotion economy, crawling is energetically more efficient than swimming [53]. Interestingly, little is known about gait specific adaptations in response to different environments. Here, we analysed the functional activity of the crawling and swimming gait in response to food deprivation in the model organism *C. elegans* using phenotypic and genetic approaches.

Our study revealed that the locomotor activity of crawling and swimming are differentially regulated in response to dietary restriction (DR). We showed, that *C. elegans* responds to DR with remarkably accelerated body bending swimming frequency (BBSF) and slightly increased speed of swimming. In contrast, locomotor activity of the crawling gait is reduced under DR in comparison to *ad libitum* (AL) condition. Furthermore, DR reduces body size and protein content of wild type worms. Correspondent, analysis of the swimming gait in viscous liquids suggested that the swimming power of dietary restricted nematodes is reduced compared to AL fed worms. By means of mutant strain analysis, altered body size and/ or body elasticity seems not to be relevant for DR induced acceleration of BBSF. Mutants deficient in the TOR, AMPK as well as mainly $G\alpha_o$ signalling pathways showed a reduced BBSF response to DR. Thus, we propose that sensing of amino acids (TOR pathway) and cellular energy status (AMPK pathway) as well as regulating neurotransmitter release ($G\alpha_o$ - $G\alpha_q$ signalling) contribute to increased swimming activity of food deprived nematodes (**table 2 and 3**).

Table 2. Summary of DR responses in *C. elegans*.

<i>responses to food deprivation</i>	<i>DR-model</i>
body bending frequency BBSF	DR ↑↑↑ Starv ↑↑↑ <i>eat-2</i> ↑↑ <i>pept-1</i> ↑↑
	responses to DR
BBCF	↓
speed of movement swimming speed	↑
crawling speed	↓
power of swimming BBSF	↓
swimming speed	↓↓
body proportion length	↓↓
volume	↓↓
body composition protein	↓↓↓
protein bound amino acids	↓↓↓
free amino acids	↓↓↓

* ↑-↑↑↑: weak to strong increase, ↓-↓↓↓: weak to strong decrease in response to DR

4.1. Data for body bending frequencies and speed of movements are in line with previously reported data

In AL fed N2 worms, we determined a body bending crawling frequency (BBCF) of 0.68 Hz which lies between reported values of 0.2 to 1.1 Hz [56, 57, 63, 64, 67, 101, 103-108]. Obtained BBSF of 1.78 Hz in AL fed N2 worms are also comparable to reported frequencies ranging between 1.4 and 2.1 Hz [54, 56, 57, 63, 64, 72, 91, 93, 101-107, 109-113]. In the present study, the speed of crawling and swimming was 0.30 mm/s and 0.33 mm/s, respectively, according to reported values between 0.1 mm/s and 0.27 mm/s for crawling and 0.12 mm/s and 0.38 mm/s for swimming [56, 63, 72, 104, 106-109]. There are only two studies examining *C. elegans* locomotory rate of crawling under different feeding conditions [67, 108], but none investigating the rate of swimming. The BBCF of well fed worms, moving on plates

lacking or containing bacteria, were 0.57 Hz and 0.68 Hz, respectively, according to reported values [67, 108] of 0.6 to 1.1 Hz and 0.3 to 0.75 Hz, respectively. Reported BBCF of short term starved worms, analysed on plates without or with bacterial lawn, was 1.1 Hz and 0.1 to 0.2 Hz, respectively [67, 108]. Our results have shown a BBCF of 0.64 Hz and 0.45 Hz for dietary restricted animals. A reason for this difference might be, that extent of exhaustion, which affects locomotion [114], differ between short term starvation and dietary restriction regimes. Another explanation of different BBCF might be, that the authors let the worms enough time to adapt to the new environment, before analysing BBCF. In our experimental set-up, films were recorded immediately after the worms were picked on the plates in order to reduce the influence of adaptive behaviour on locomotory rate and to obtain data comparable to the swimming assay.

Table 3. Influence of genetic models on increased BBSF in response to DR.

<i>genetic model</i>	<i>impact on BBSF response to DR</i>
body size	-
body elasticity	-
neuropeptides - and receptors	-
metabotropic glutamate receptors	-
TOR	↑
AMPK	↑↑
G-protein- and $G\alpha_o$ - $G\alpha_q$ signaling network	↑↑↑

* - : no impact, ↑ - ↑↑↑: weak to strong impact

4.2. In contrast to the crawling gait, the activity of the swimming gait is independent of bacterial food being present or lacking in the assay medium

Previous studies have shown that crawling activity is affected by many environmental stimuli and is substantially influenced by food seeking strategies. For example,

C. elegans can alternate locomotion between roaming and dwelling mode depending on sensory perception [103]. As found in *C. elegans* [67] as well as in other free-living rhabditids [108], well-fed nematodes that were transferred to assay plates containing bacterial food crawled more slowly than well-fed animals entering assay plates lacking bacteria. This so called basal slowing response, which we could also observe in our experimental set-up, is more pronounced in worms exposed to short term (30 min) starvation. Consistent with this, we also observed an enhanced slowing response in terms of crawling frequency and crawling speed, respectively, in dietary restricted worms. In contrast, a basal slowing response as well as an enhanced slowing response was not found in AL or DR fed worms that were transferred to liquid assay media containing or lacking bacteria. Thus, in contrast to the crawling gait, the activity of the swimming gait is independent of bacterial food, emphasizing that food restriction induces gait specific effects.

4.3. Acceleration of locomotory rate in response to dietary restriction is specific for the swimming gait and seems to be of adaptive significance

Consistent with reported data [67, 108], DR reduces the activity of the crawling gait. Unexpectedly, we found that *C. elegans* wild type worms increase locomotory rate of swimming markedly in response to DR. Dietary restricted worms exhibit a 53 % higher BBSF compared to AL fed animals. Accordingly, the resulting swimming speed was slightly increased under DR condition. Moreover, the swimming economy, calculated by the ratio between the worm's forward speed and the speed of its body waves, was also slightly increased in dietary restricted worms. Thus, accelerated BBSF in response to DR is translated into forward movement. Because nematode's swimming is less efficient than crawling [53], the identified swimming response is important to maintain or even increase swimming speed of nematodes upon nutrient limitation to direct their motion towards favourable crawling conditions. Therefore, we propose that the DR induced acceleration of BBSF and swimming speed is of adaptive significance. In line with this, BBSF of adult worms, exposed to DR during development and a subsequent AL re-feeding period of 24-96 h, was higher than BBSF of worms permanently exposed to AL. This persistent acceleration of BBSF may reflect that *C. elegans* can adopt alternative adult life histories [115], a long-term adaptation to different environmental conditions. Interestingly, calculation of swimming speed per body length revealed that the relative speed of swimming is

about 2-fold higher in DR worms compared to AL fed animals. However, the adaptive significance of this allometric consideration remains to be elucidated.

4.4. The small body size of dietary restricted worms per se seems not to be important to explain DR induced acceleration of BBSF

Since locomotion of nematodes is characterized by low Reynolds number, their movement is substantially influenced by environmental factors [100]. This biomechanical consideration implies that body size and body elasticity are less important physical constraints of the worm's locomotory activity. In line with this, mutants (*lon-2*, *egl-4*, *che-2*) with altered body length, width or elasticity showed similar BBSF under AL condition than wild type. Thus, the small body size of dietary restricted worms per se seems not to be important to explain DR induced acceleration of BBSF. Rather, the swimming response is likely a specific physiological or metabolic response.

4.5. Acceleration of BBSF is inducible by quite different DR regimes

As discussed previously [116], different DR regimes lead to overlapping but not identical responses in *C. elegans*. In the present study, we found that acceleration of BBSF was inducible by quite different DR regimes including developmental DR, which was recently developed in our lab [74], genetic models of DR and starvation, initiated at L4 and young adult stage. Because our standard DR regime as well as genetic models of DR implied a long-term food deprivation during development [74], the impact of short-term starvation on BBSF was examined. We found a positive relationship between acceleration of BBSF and time of starvation suggesting that the extent of exhaustion is an important signal for the modulatory response.

4.6. Muscle protein depletion might be an important signal for accelerated locomotor activity in response to DR

Compared to AL fed worms, the protein content of dietary restricted nematodes was reduced by about 40 %. In mammals, muscle proteins represent nearly half of the total protein pool [117]. Assuming similar proportions in *C. elegans*, we postulated that dietary restricted worms contain less muscular mass than AL fed worms. In line

with this, starvation activates the proteolytic system and leads to degradation of body wall muscles in *C. elegans* [113, 118, 119]. Moreover, we found that the decline of swimming frequency and speed by increasing liquid viscosity is higher for food deprived worms than for well fed ones. This suggested that muscle strength of DR fed worms is decreased. In response to DR we could show, that the protein content was reduced, suggesting a less muscle mass and less muscle strength, which is despite combined with higher locomotory activity. This is reminiscent to a phenomenon described in penguins and rats. Penguins end their long fasting period while breeding, when the fat stores are depleted and protein utilization increases. Then the starved birds start walking and re-feeding. [120-122] In starved rats, the rise in locomotor activity is also related with increased protein utilization. [120] Therefore, utilization of muscle protein might be an internal signal inducing locomotor activity, a evolutionary conserved mechanism present in animals from diverse taxa including *C. elegans*.

4.7. Sensing of intracellular amino acids and energy status via the TOR and AMPK pathway might contribute to the accelerated BBSF in response to dietary restriction

The *pept-1* mutant strain is a genetic model of protein restriction, because of its impairment in absorption of di-/ tripeptide-bound amino acids in the intestine [30]. According to our hypothesis that protein depletion contributes to locomotory rate, *pept-1* mutant worms showed higher BBSF even under AL feeding condition. The TOR pathway senses intracellular amino acid availability [123, 124] and regulates the function of the peptide transporter PEPT-1 [30] and other amino acid transporter [125, 126]. Interestingly, mutants (*rikt-1*, *sgk-1*, *akt-1*) lacking components of the non-canonical TOR complex 2 (TORC2) showed a minor BBSF response to DR than N2 worms did. Furthermore, mutant strains (*aak-2*) deficient in the AMP-activated protein kinase (AMPK) pathway showed a minor response, too. It has been shown, that the AMPK pathway acts as an intracellular energy sensor and it is activated by caloric restriction and was found to be important in motor neuron processes [91]. Thus we propose that intracellular amino acids and energy status contribute, at least in part, to the DR induced acceleration of BBSF. In line with this, the concentration of several non-protein bound (free) amino acids including leucin, an activator of the TOR pathway [124, 127], were altered in response to DR. The relationship between dietary

amino acids, intracellular amino acids, muscle protein content, TOR pathway and swimming activity remains to be examined.

4.8. Statistical analysis of data with respect to multiple comparisons revealed that *gbp-2*, *ser-3*, *dgk-1*, *goa-1* and *egl-8* mutant strains contribute to the swimming response

Statistical analyses of data, obtained by explorative and experimental studies, are usually performed using Student's T-test or one-way analysis of variance (ANOVA) combined with post-hoc tests. We also used one-way ANOVA with Bonferroni post-hoc test to analyse our data. However, advanced statistical analysis with respect to multiple comparisons was additionally performed, because a lot of mutants were analysed in independent experiments. For this purpose, BBSF of each mutant was normalized to those frequencies, obtained from AL fed wild type worms of the same experiment. This was done to minimize variations which occur in the laboratory praxis from experiment to experiment. Initially, we used our statistical model to find out if the mutant strains do significantly response to DR compared to AL condition. A significant ($p < 0.001$) acceleration of BBSF to DR was found in almost all mutant strains. Next, our statistical model was used to investigate which mutant strain shows the weakest respond to DR. Thus, we wanted to find out which mutant strain shows the most equal BBSF obtained under AL and DR condition. If accepting a maximal difference of 20 % between BBSF under AL and DR condition, mutant strains *gbp-2* (*ad541*) and *ser-3* (*ad1774*) were identified using an equivalence test. *Dgk-1* (*nu62*), *goa-1* (*n1134*) and *egl-8* (*md1971*) deficient mutant showed differences lower than 28 %. The TORC2 related mutant strains (*akt-1*, *sgk-1*, *rict-1*) showed a difference between 33-38 %. Statistical equivalence analysis of the AMPK mutant strains *aak-2* revealed differences about 30-35 %. For details see **table S7**. Overall, conventional as well as more accurate statistical analysis revealed that *gbp-2* (*ad541*), *ser-3* (*ad1774*), *dgk-1* (*nu62*), *goa-1* (*n1134*) and *egl-8* (*md1971*) mutant strains might contribute to the BBSF response, whereas an involvement of *akt-1* (*mg306*), *sgk-1* (*ft15*), *rict-1* (*ft7* and *mg360*) and *aak-2* (*ok524* and *rr48*) remains to be examined.

4.9. The interacting EGL-30 ($G\alpha_q$) and GOA-1 ($G\alpha_o$) signalling network is involved in mediating the DR induced increase of BBSF

Compared to wild type, mutants lacking components of the interacting EGL-30 ($G\alpha_q$) and GOA-1 ($G\alpha_o$) pathway, *goa-1* (*n1134*), *dgk-1* (*nu62*), *egl-8* (*md1971*) and *gbp-2* (*ad541* and *pk751*), showed similar BBSF at AL feeding condition but exhibited a less BBSF response to DR. In which the GOA-1 ($G\alpha_o$) pathway revealed to be mainly involved in mediating the DR induced increase of BBSF. Interaction of these two evolutionary conserved pathways regulates acetylcholine release from motor neurons via modulating diacylglycerol (DAG) levels. The central regulator of these pathways, GBP-2, is a G-protein β -subunit that interacts with EGL-10 and EAT-16 to inhibit EGL-30 and GOA-1 activity and is expressed in neurons and muscle cells [63]. *C. elegans* GBP-2 is related to mammalian $G\beta 5$ -subunit that functions as a regulator of G-protein signalling, which can act as GTPase-activating protein to inhibit $G\alpha_o$ activity. Remarkably, *gbp-2* mutant strain showed a) almost no swimming response, in terms of body bending frequency and speed, to DR, b) no basal and enhanced slowing response regarding BBSF and c) a reduced basal and enhanced slowing response regarding speed of crawling. This suggested, that GBP-2 plays a central role, via EGL-30 ($G\alpha_q$) and GOA-1 ($G\alpha_o$), for locomotor responses to DR. In line with this hypothesis, it has been shown in *C. elegans* that GBP-2 is necessary for survival of the animals under starvation [128].

4.10. Octopamine and serotonin might be involved in the swimming response to DR

Compared to all mutants tested, beside *gbp-2*, mutant strain *ser-3* (*ad1774*), showed the weakest BBSF response to DR. *Ser-3* encodes a G-protein coupled receptor, which functions upstream of the EGL-30 ($G\alpha_q$) and GOA-1 ($G\alpha_o$) pathway and is activated by octopamine, a biogenic amine in *C. elegans*. In the absence of food, SER-3 mediates different behavioural aspects via EGL-30 and the transcription factor CREB (cAMP response element-binding protein). In the presence of food, SER-3 is required for serotonin mediated inhibition of movement via activation of the GOA-1 pathway [67, 95, 129, 130]. Interestingly, the serotonin deficient mutant strain *tph-1* showed an un-coordinated swimming behaviour with only few regular body bends. It is also known from literature that a lack of serotonin reduces swimming activity of the

worms [57]. Therefore, we hypothesize that octopamine and serotonin are involved in the increased swimming activity in response to DR via SER-3 and GOA-1. The importance of serotonin for locomotor activity is emphasized by studies in rats. It has been shown, that starved rats exhibited hyperactivity in response to starvation. This response was found to be combined with an altered serotonin metabolism in the hypothalamus [131-133].

4.11. Adaptation of locomotor activity in response to limited food supply is an evolutionary conserved mechanism

This study revealed that *C. elegans* adapts locomotor activity upon nutrient limitation to increase body bending frequency and speed, specifically for the swimming gait. With respect to the crawling gait, DR reduces locomotion of the worms. On the basis of previous publications, we might expect that adapting the locomotor activity in response to changing food supply is an evolutionary conserved mechanism. For example, *E. coli* controls its swimming velocity by adjusting the intracellular concentration of the second messenger cyclic di-GMP. But contrary to *C. elegans*, *E. coli* slows its swimming speed when the nutrient supply decreases, entering the stationary phase [134]. A prolonged starvation of whiteleg shrimps also leads to decreased swimming speed [49]. Comparative studies in fed and starved largemouth bass fishes also revealed a decreased swimming speed and metabolic rate of starved fishes [114]. In contrast, studies on starved Wistar rats have shown hyperactive wheel running behaviour in response to starvation [120, 135]. Caloric restriction was also found to increase the spontaneous physical activity in young (4-6 month) and older (18-24 month) obesity-prone, obesity resistant and Sprague-Dawley rats [136, 137]. Caloric restriction of rhesus monkeys has led to increased activity patterns [138]. But it has to be mentioned, that locomotor activity of higher animals is hard to analyse because of the increasing influence of behavioural effects due to a higher developed consciousness. However, it has also been observed that up to 80 % of human patients with the eating disorder anorexia nervosa show excessive physical activity [120, 139]. The reasons for the paradox relationship between increasing locomotor activity and anorexia nervosa are discussed since Gull 1888 first described this eating disorder [140]. But it remains still unclear if the hyperactivity of those patients is just a strategy to loose weight or is derived by an unconscious biological need to forage or even to regulate hypothermia [139, 141].

Taken together, adaptation of locomotor activity in response to limited food supply is an evolutionary conserved phenomenon.

Based on the results of our study combined with literature data, we propose two basic behavioural modes of locomotion in response to food deprivation with adaptive significance. First, decreased locomotor activity, as described for bacteria, shrimps and fishes, might be the best strategy to save energy in order to survive in times of food restriction. Those species behave passive, because waiting until the food situation improves might be more effective than enhance foraging. Second, increasing locomotor activity is used to enhance foraging and to enhance the chance to survive like it is found in penguins, rodents and monkeys. Here, it might be more effective to primarily invest energy to increase foraging activity than to save energy. Our study revealed an increased locomotor activity in *C. elegans* exclusively for the swimming gait. What implies, that the best survival strategy for food deprived worms exposed to liquids is to direct motion fast towards favourable conditions.

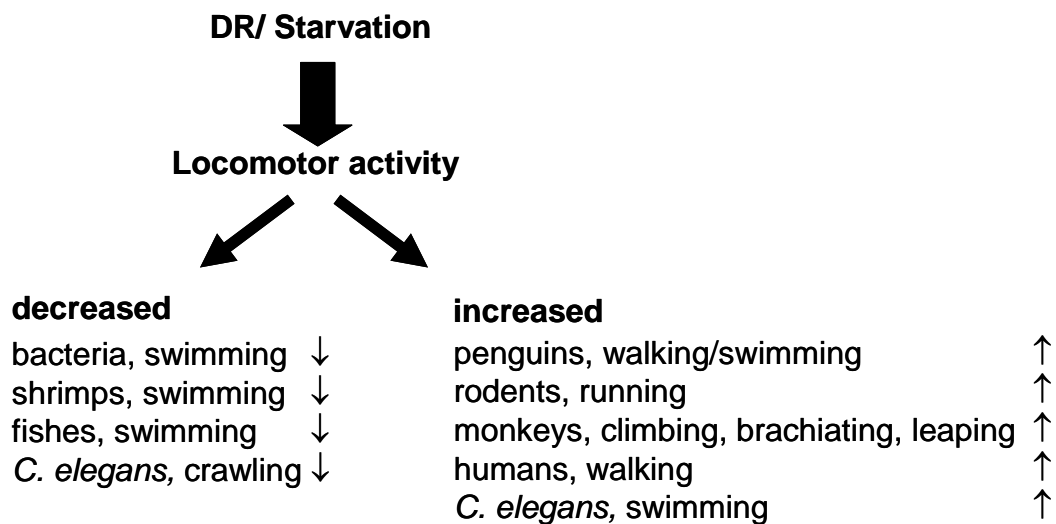


Figure 12. Summary of DR induced responses on locomotory activity.

5. Supplementary Information

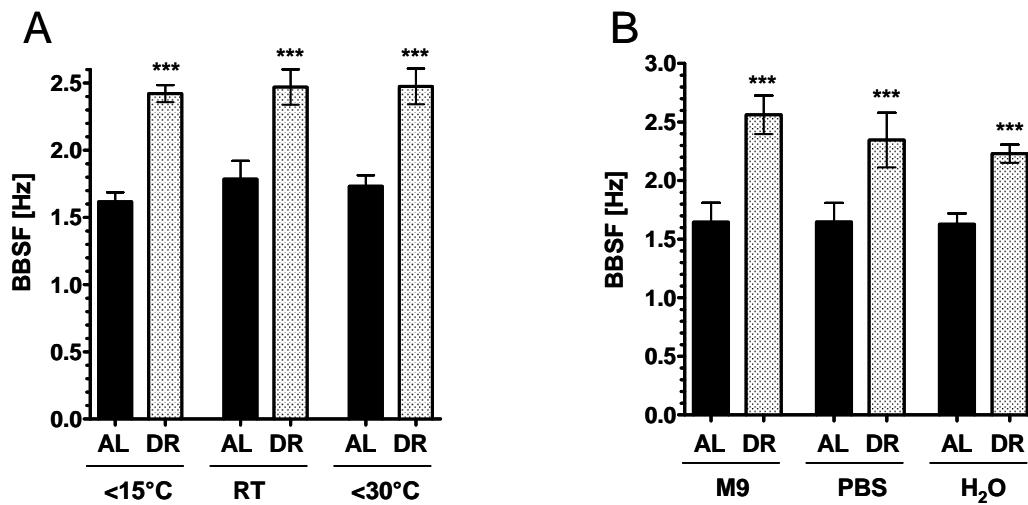


Figure S1. Influence of temperature and osmolarity of the assay medium on body bending swimming frequency of adult wild type worms.

A) Temperature of the assay medium (M9 buffer) was cooled to approximately 15 °C or heated to approx. 30°C. Results of BBSF (in Hz) are shown as mean (\pm SD) of N = 3 experiments and n = 10-26 counted worms per condition. Statistical comparison of AL vs. DR at same temperatures is illustrated. Comparison of BBSF at room temperature (RT) vs. 15°C vs. 30°C under the same feeding condition results in no significant differences, not illustrated.

B) Influence of different assay media on BBSF of AL fed and dietary restricted adult wild type worms. Adult N2 were picked in M9 buffer, PBS buffer or water, respectively. N = 3 with n = 14-26 worms per condition have been analysed. Statistical comparison of AL vs. DR condition, swimming in the same liquid are illustrated. Comparison of different media at same feeding conditions results in no significant differences.

A), B) One-way ANOVA was performed and Bonferroni multiple comparison test was used as post test ($p > 0.05$, $***p < 0.001$).

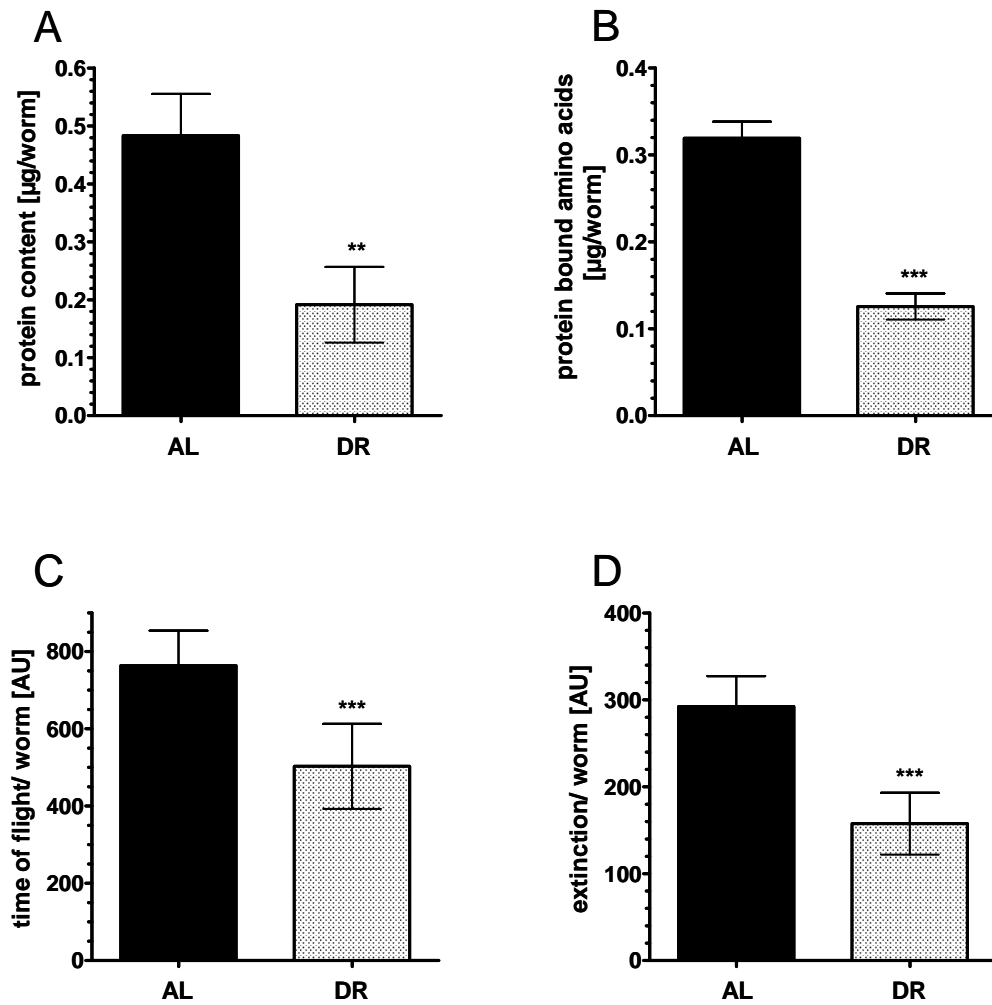


Figure S2. Protein content and body proportion of *ad libitum* and dietary restricted adult wild type worms.

Protein content of AL and DR fed worms analysed by fluorometric assay (A) and determination of protein bound amino acids (B) confirmed results of bicinchoninic acid (BCA) protein assay (see Fig. 8C).

Time of flight (C) and extinction (D) values indicating body length and body volume of the worms, respectively, analysed by the flow-cytometry based COPAS Biosort system. A)-D) depicts means (\pm SD) of N = 3 independent experiments with n = 8654 AL worms and n = 23124 DR worms in total. A) Significant results analysed by one-way ANOVA and Bonferroni multiple comparison as post test (** $p < 0.01$). B)-D) were analysed by Student's T-Test (*** $p < 0.001$).

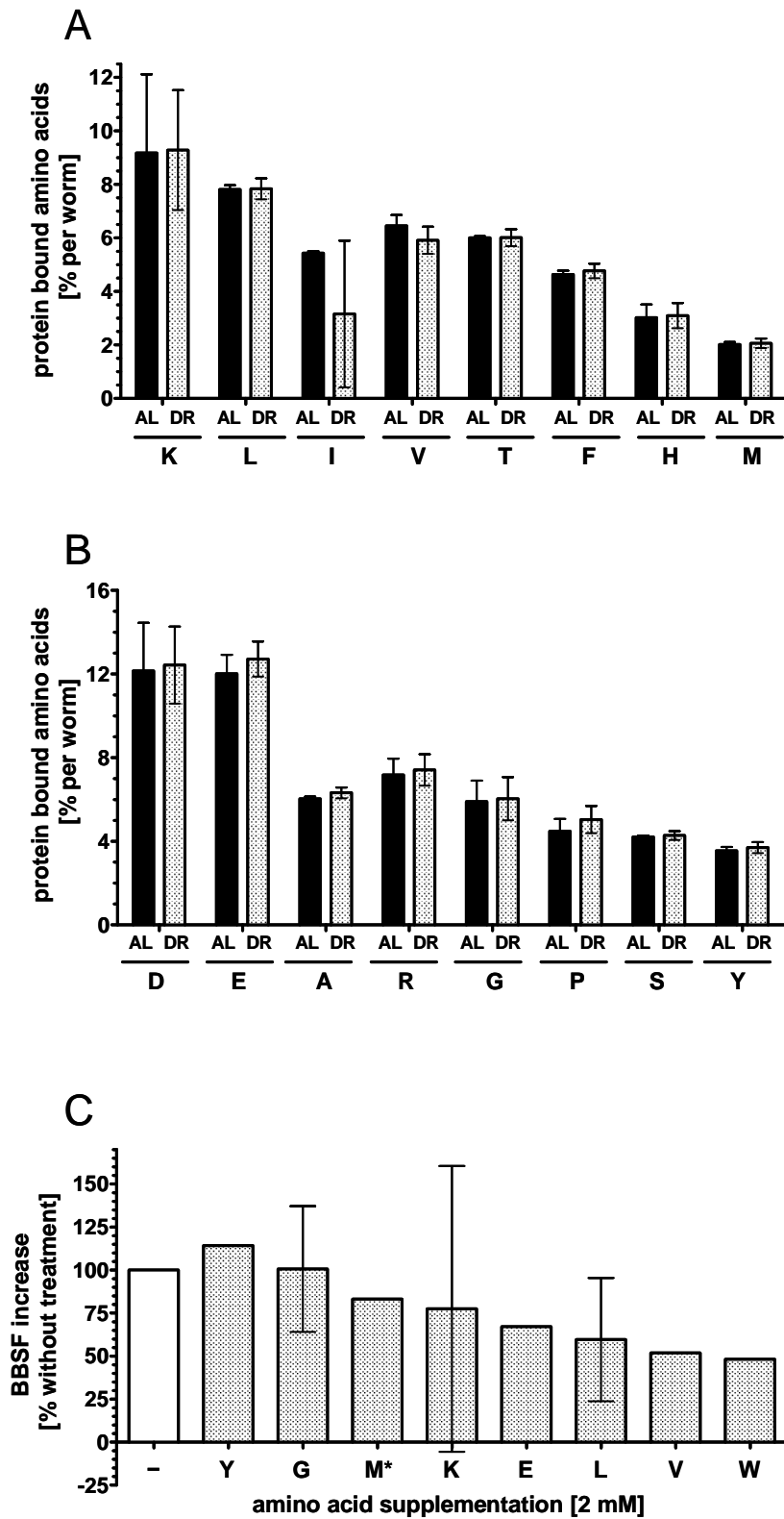


Figure S3. Protein bound amino acid content of *ad libitum* fed and dietary restricted adult wild type worms and effect of amino acid supplementation on DR induced acceleration of BBSF of wild type worms.

Relative proportion of essential (A) and non-essential (B) protein bound amino acids of AL fed (black) and dietary restricted (grey) adult wild type worms. The mean values (\pm SD) of

N= 3 independent experiments with n = 8654 AL worms and n = 23124 DR worms in total are shown.

C) BBSF of dietary restricted adult wild type worms supplemented with indicated amino acids, expressed as percent of BBSF increase due to DR compared to the untreated AL control group. Results were normalized to BBSF increase of untreated DR group. Wild type worms were supplemented with amino acid dilutions spotted on DR plates from egg to adulthood to reach a final concentration of 2 mM (*except for methionine: 1 mM). N = 1-4 with n = 3-6 worms analysed per experiment and condition. **A)-C)** One-way ANOVA and Bonferroni multiple comparison as post test was used for statistical comparison of AL vs. DR condition (**A,B**) and treated DR (grey) vs. not treated DR (white) (**C**) adult wild type ($p > 0.05$).

Table S1. Original data of BBSF mutant screen analysis.

Resulting BBSF of all tested adult mutant strains illustrated as mean of all analysed experiments as bb/30 s at AL and DR feeding condition.

<i>Strain</i>	<i>Ad libitum feeding (body bends/ 30 s)</i>	<i>Standard Deviation SD (±)</i>	<i>Number of counted worms (n)</i>	<i>Dietary restriction (body bends/ 30 s)</i>	<i>Standard Deviation SD (±)</i>	<i>Number of counted worms (n)</i>	<i>Number of experiments (N)</i>
<i>N2 (Bristol)</i>	50.41	4.98	297	77.49	4.32	283	49
<i>aak-2 (ok524)</i>	52.41	4.30	15	62.40	2.51	14	3
<i>aak-2 (rr48)</i>	52.54	4.42	14	65.12	5.68	15	3
<i>acr-2 (ok1887)</i>	58.60	0.00	5	74.80	0.00	5	1
<i>akt-1 (mg306)</i>	56.33	0.93	18	67.28	1.40	18	3
<i>cat-2 (e1112)</i>	58.57	7.40	11	76.93	3.21	11	2
<i>che-2 (e1033)</i>	55.29	2.14	24	69.33	5.23	22	4
<i>col-179 (ok3010)</i>	61.77	5.49	59	73.70	6.27	54	11
<i>col-61 (ok2582)</i>	54.17	0.00	6	78.00	0.00	6	1
<i>col-99 (ok1204)</i>	52.50	0.00	6	70.17	0.00	6	1
<i>daf-22 (ok693)</i>	51.44	1.49	24	62.53	1.88	19	3
<i>daf-7 (e1372)</i>	56.94	11.26	15	66.89	7.04	15	3
<i>dgk-1 (nu62)</i>	54.96	3.86	45	60.50	3.70	44	7
<i>dpy-5 (e61)</i>	62.20	0.00	5	75.20	0.00	5	1
<i>eat-16 (sa609)</i>	47.17	1.18	12	59.58	1.53	12	2
<i>eat-2 (ad465)</i>	62.43	1.67	21	75.70	11.19	21	3
<i>egl-30 (ad805)</i>	0.00	0.00	6	0.00	0.00	6	1
<i>egl-4 (n478)</i>	53.17	0.24	12	76.50	1.41	10	2
<i>egl-8 (e2917)</i>	49.00	0.95	24	58.75	2.11	24	4
<i>egl-8 (md1971)</i>	51.71	3.97	24	56.77	2.21	25	4
<i>far-4 (ok317)</i>	50.50	0.00	6	81.17	0.00	6	1
<i>flp-18 (gk3063)</i>	41.83	0.00	6	56.50	0.00	6	1
<i>gbp-2 (ad541)</i>	60.15	2.17	57	63.78	4.38	55	10
<i>gbp-2 (pk751)</i>	63.78	1.67	18	69.72	3.68	18	3
<i>goa-1 (n1134)</i>	53.08	5.61	42	60.84	5.54	42	7
<i>goa-1 (syls9)</i>	0.00	0.00	6	0.00	0.00	6	1
<i>lon-2 (e678)</i>	52.72	3.24	16	65.83	3.50	16	3
<i>mec-4 (e1611)</i>	59.80	0.00	5	81.20	0.00	5	1

Strain	Ad libitum feeding (body bends/ 30 s)	Standard Deviation SD (\pm)	Number of counted worms (n)	Dietary restriction (body bends/ 30 s)	Standard Deviation SD (\pm)	Number of counted worms (n)	Number of experiments (N)
<i>mgl-1 (tm1811)</i>	48.93	2.34	17	63.61	1.06	17	3
<i>mgl-2 (tm355)</i>	39.51	2.41	23	51.57	8.36	26	4
<i>npr-4 (tm1782)</i>	46.33	0.47	11	67.04	0.41	10	2
<i>npr-5 (ok1583)</i>	54.83	0.00	6	68.50	0.00	6	1
<i>odr-1 (n1936)</i>	57.00	0.00	3	74.67	0.00	3	1
<i>pGK10 ::GFP</i>	45.00	1.41	8	64.63	1.59	8	2
<i>raga-1 (ok386)</i>	56.50	0.00	4	74.67	0.00	3	1
<i>rict-1 (ft7)</i>	51.33	5.72	24	68.75	2.47	24	4
<i>rict-1 (mg360)</i>	53.86	4.24	18	62.39	6.56	18	3
<i>rsk-1 (ok1255)</i>	54.25	0.00	4	69.75	0.00	4	1
<i>ser-2 (pk1357)</i>	52.42	3.89	12	70.92	5.54	12	2
<i>ser-3 (ad1774)</i>	64.21	4.06	59	68.78	5.50	57	11
<i>sgk-1 (ft15)</i>	52.22	2.98	18	60.73	0.40	18	3
<i>snf-1 (ok790)</i>	56.27	6.98	11	67.38	5.96	11	2
<i>srp-3 (ok1433)</i>	50.22	0.00	9	80.60	0.00	5	1
<i>tph-1 (mg280)</i>	28.33	0.00	3	35.33	0.00	3	1
<i>trp-4 (sy695)</i>	54.58	1.59	19	71.17	2.00	16	4
<i>unc-63 (x37)</i>	1.78	0.00	15	6.00	0.00	13	1

Table S2. Statistical analysis of BBSF of AL and DR wild type and mutant strain adults swimming either in liquids with (+) or without (-) bacteria.

Statistics were performed as described in 2.5. by two-way ANOVA and 95 % coverage probability, concerning comparison of feeding condition, genotype and bacterial food in liquid.

Strain	Feeding condition	Bacteria in liquid	p-value	Estimate	Standard Error	lower level	upper level
Influence of the feeding condition - dietary restriction compared to AL feeding							
N2	DR vs. AL	-	0.0039	34.89	6.33	12.63	57.15
N2	DR vs. AL	+	0.0033	36.71	6.47	13.94	59.48
<i>dgk-1 (nu62)</i>	DR vs. AL	-	0.9787	5.62	7.35	-20.25	31.49
<i>dgk-1 (nu62)</i>	DR vs. AL	+	0.9952	4.48	7.45	-21.74	30.71
<i>egl-8 (md1971)</i>	DR vs. AL	-	0.0444	-30.00	8.32	-59.27	-0.72
<i>egl-8 (md1971)</i>	DR vs. AL	+	0.0111	43.39	9.31	10.65	76.13
<i>gbp-2 (ad541)</i>	DR vs. AL	-	0.9998	2.84	7.31	-22.87	28.55
<i>gbp-2 (ad541)</i>	DR vs. AL	+	1.0000	-1.83	7.50	-28.22	24.57
<i>ser-3 (ad1774)</i>	DR vs. AL	-	0.9973	4.08	7.40	-21.94	30.10
<i>ser-3 (ad1774)</i>	DR vs. AL	+	0.9996	3.22	7.45	-23.01	29.44
Influence of the genotype compared to N2							
<i>dgk-1 (nu62)</i>	AL	-	0.9623	5.43	4.28	-7.23	18.08
<i>dgk-1 (nu62)</i>	AL	+	0.9275	5.90	4.28	-6.75	18.55
<i>dgk-1 (nu62)</i>	DR	-	< 0.0001	-23.85	4.20	-36.28	-11.42
<i>dgk-1 (nu62)</i>	DR	+	< 0.0001	-26.32	4.28	-38.97	-13.67
<i>egl-8 (md1971)</i>	AL	-	0.2680	-12.16	5.23	-27.64	3.32
<i>egl-8 (md1971)</i>	AL	+	0.0028	-23.89	6.29	-42.50	-5.27
<i>egl-8 (md1971)</i>	DR	-	< 0.0001	-77.05	5.01	-91.86	-62.24
<i>egl-8 (md1971)</i>	DR	+	0.0725	-17.20	6.07	-35.17	0.76
<i>gbp-2 (ad541)</i>	AL	-	0.0005	17.77	4.20	5.34	30.20
<i>gbp-2 (ad541)</i>	AL	+	0.0001	20.01	4.36	7.12	32.91
<i>gbp-2 (ad541)</i>	DR	-	0.0119	-14.28	4.20	-26.71	-1.86
<i>gbp-2 (ad541)</i>	DR	+	0.0003	-18.52	4.28	-31.17	-5.87
<i>ser-3 (ad1774)</i>	AL	-	0.0002	19.15	4.28	6.50	31.81
<i>ser-3 (ad1774)</i>	AL	+	0.4387	8.87	4.28	-3.78	21.52
<i>ser-3 (ad1774)</i>	DR	-	0.0983	-11.66	4.28	-24.31	1.00
<i>ser-3 (ad1774)</i>	DR	+	< 0.0001	-24.62	4.28	-37.27	-11.97
Influence of liquid containing bacteria (+) compared to M9 buffer (-)							
N2	AL	+ vs. -	0.9955	-3.53	6.43	-32.96	25.91
N2	DR	+ vs. -	1.0000	-1.71	6.42	-31.10	27.68
<i>dgk-1 (nu62)</i>	AL	+ vs. -	0.9994	-3.05	7.45	-37.16	31.06
<i>dgk-1 (nu62)</i>	DR	+ vs. -	0.9945	-4.19	7.40	-38.07	29.70
<i>egl-8 (md1971)</i>	AL	+ vs. -	0.5746	-15.26	8.97	-56.34	25.83
<i>egl-8 (md1971)</i>	DR	+ vs. -	0.0135	58.13	8.72	18.21	98.05
<i>gbp-2 (ad541)</i>	AL	+ vs. -	1.0000	-1.29	7.45	-35.41	32.84
<i>gbp-2 (ad541)</i>	DR	+ vs. -	0.9626	-5.95	7.40	-39.84	27.94
<i>ser-3 (ad1774)</i>	AL	+ vs. -	0.5049	-13.81	7.45	-47.91	20.30
<i>ser-3 (ad1774)</i>	DR	+ vs. -	0.4553	-14.67	7.44	-48.76	19.41

Table S3. Statistical analysis of BBCF of AL and DR wild type and mutant strain adults either crawling on plates with (+) or without (-) bacteria.

Statistics were performed as described in 2.5. by two-way ANOVA and 95 % coverage probability, concerning comparison of feeding condition, genotype and bacterial food on plates.

Strain	Feeding condition	Bacteria on plate	p-value	Estimate	Standard error	lower level	upper level
Influence of the feeding condition - dietary restriction compared to AL feeding							
N2	DR vs. AL	-	0.58213	-6.70	4.41	-20.35	6.94
N2	DR vs. AL	+	0.00859	-18.22	4.47	-32.04	-4.39
egl-8 (md1971)	DR vs. AL	-	0.58071	-9.92	6.53	-30.10	10.25
egl-8 (md1971)	DR vs. AL	+	1	-0.81	7.88	-25.17	23.55
gbp-2 (ad541)	DR vs. AL	-	0.48837	9.55	5.72	-8.14	27.24
gbp-2 (ad541)	DR vs. AL	+	0.46523	9.88	5.79	-8.01	27.78
Influence of the genotype compared to N2							
egl-8 (md1971)	AL	-	< 0.00001	-33.21	5.50	-48.34	-18.09
egl-8 (md1971)	AL	+	< 0.00001	-33.94	5.64	-49.45	-18.44
egl-8 (md1971)	DR	-	< 0.00001	-36.43	5.30	-50.99	-21.88
egl-8 (md1971)	DR	+	0.113187	-16.54	6.76	-35.10	2.03
gbp-2 (ad541)	AL	-	0.14205	11.30	4.79	-1.88	24.47
gbp-2 (ad541)	AL	+	0.000955	18.53	4.74	5.51	31.55
gbp-2 (ad541)	DR	-	< 0.00001	27.55	4.73	14.54	40.56
gbp-2 (ad541)	DR	+	< 0.00001	46.63	4.93	33.09	60.18
Influence of bacteria lawn on plates (+) compared to empty plates (-)							
N2	AL	+ vs. -	0.09852	-16.11	5.29	-35.27	3.05
N2	DR	+ vs. -	0.00978	-27.62	5.34	-46.95	-8.29
egl-8 (md1971)	AL	+ vs. -	0.26111	-16.84	7.53	-44.07	10.39
egl-8 (md1971)	DR	+ vs. -	0.89029	-7.73	8.26	-37.60	22.15
gbp-2 (ad541)	AL	+ vs. -	0.67494	-8.87	6.57	-32.66	14.91
gbp-2 (ad541)	DR	+ vs. -	0.71104	-8.54	6.64	-32.54	15.47

Table S4. Statistical analysis of swimming speed of AL and DR wild type and mutant strain adults swimming either in liquids with (+) or without (-) bacteria.

Statistics were performed as described in 2.5. by two-way ANOVA and 95 % coverage probability, concerning comparison of feeding condition, genotype and bacterial food in liquid.

Strain	Feeding condition	Bacteria in liquid	p-value	Estimate	Standard Error	lower level	upper level
Influence of the feeding condition - dietary restriction compared to AL feeding							
N2	DR vs. AL	-	0.448	14.20	7.18	-10.58	38.98
N2	DR vs. AL	+	0.056	24.55	7.27	-0.54	49.65
<i>dgk-1 (nu62)</i>	DR vs. AL	-	1.000	0.85	10.33	-34.78	36.49
<i>dgk-1 (nu62)</i>	DR vs. AL	+	1.000	-0.39	9.85	-34.38	33.61
<i>egl-8 (md1971)</i>	DR vs. AL	-	1.000	-0.32	10.44	-36.35	35.71
<i>egl-8 (md1971)</i>	DR vs. AL	+	0.620	16.85	9.98	-17.59	51.29
<i>gbp-2 (ad541)</i>	DR vs. AL	-	0.880	-11.02	8.97	-41.98	19.94
<i>gbp-2 (ad541)</i>	DR vs. AL	+	0.426	-17.74	8.79	-48.08	12.61
<i>ser-3 (ad1774)</i>	DR vs. AL	-	0.879	-12.27	9.97	-46.69	22.15
<i>ser-3 (ad1774)</i>	DR vs. AL	+	1.000	2.61	9.85	-31.38	36.61
Influence of the genotype compared to N2							
<i>dgk-1 (nu62)</i>	AL	-	0.948	-11.01	8.32	-35.64	13.63
<i>dgk-1 (nu62)</i>	AL	+	1.000	4.06	7.72	-18.77	26.89
<i>dgk-1 (nu62)</i>	DR	-	0.027	-24.35	7.72	-47.19	-1.51
<i>dgk-1 (nu62)</i>	DR	+	0.121	-20.88	7.88	-44.19	2.44
<i>egl-8 (md1971)</i>	AL	-	0.000	-36.14	8.30	-60.70	-11.57
<i>egl-8 (md1971)</i>	AL	+	<0.0001	-35.84	7.72	-58.67	-13.01
<i>egl-8 (md1971)</i>	DR	-	<0.0001	-50.66	7.90	-74.04	-27.28
<i>egl-8 (md1971)</i>	DR	+	<0.0001	-43.55	7.92	-66.99	-20.11
<i>gbp-2 (ad541)</i>	AL	-	0.117	18.95	7.12	-2.12	40.03
<i>gbp-2 (ad541)</i>	AL	+	<0.0001	34.18	6.90	13.78	54.59
<i>gbp-2 (ad541)</i>	DR	-	0.999	-6.27	7.12	-27.33	14.80
<i>gbp-2 (ad541)</i>	DR	+	0.988	-8.11	7.25	-29.57	13.35
<i>ser-3 (ad1774)</i>	AL	-	0.207	18.81	7.72	-4.03	41.65
<i>ser-3 (ad1774)</i>	AL	+	0.743	13.12	7.72	-9.71	35.95
<i>ser-3 (ad1774)</i>	DR	-	0.997	-7.66	7.88	-30.99	15.67
<i>ser-3 (ad1774)</i>	DR	+	0.988	-8.82	7.88	-32.13	14.50
Influence of liquid containing bacteria (+) compared to M9 buffer (-)							
N2	AL	+ vs. -	0.732	-10.85	7.23	-41.80	20.10
N2	DR	+ vs. -	1.000	-0.49	7.32	-31.83	30.85
<i>dgk-1 (nu62)</i>	AL	+ vs. -	1.000	4.22	10.37	-40.18	48.63
<i>dgk-1 (nu62)</i>	DR	+ vs. -	1.000	2.98	9.90	-39.41	45.37
<i>egl-8 (md1971)</i>	AL	+ vs. -	0.939	-10.56	10.35	-54.88	33.77
<i>egl-8 (md1971)</i>	DR	+ vs. -	0.996	6.62	10.16	-36.92	50.15
<i>gbp-2 (ad541)</i>	AL	+ vs. -	0.999	4.38	8.84	-33.48	42.25
<i>gbp-2 (ad541)</i>	DR	+ vs. -	1.000	-2.34	9.01	-40.94	36.27
<i>ser-3 (ad1774)</i>	AL	+ vs. -	0.642	-16.54	9.89	-58.88	25.81
<i>ser-3 (ad1774)</i>	DR	+ vs. -	1.000	-1.65	10.03	-44.60	41.30

Table S5. Statistical analysis of crawling speed of AL and DR wild type and mutant strain adults either crawling on plates with (+) or without (-) bacteria.

Statistics were performed as described in 2.5. by two-way ANOVA and 95 % coverage probability, concerning comparison of feeding condition, genotype and bacterial food on plates.

<i>Strain</i>	<i>Feeding condition</i>	<i>Bacteria on plates</i>	<i>p-value</i>	<i>Estimate</i>	<i>Standard Error</i>	<i>lower level</i>	<i>upper level</i>
Influence of the feeding condition - dietary restriction compared to AL feeding							
N2	DR vs. AL	-	< 0.001	-39.77	4.69	-54.30	-25.25
N2	DR vs. AL	+	< 0.001	-52.90	4.63	-67.21	-38.59
egl-8 (md1971)	DR vs. AL	-	0.0691	-18.31	6.28	-37.75	1.14
egl-8 (md1971)	DR vs. AL	+	0.9993	-2.43	6.85	-23.62	18.76
gbp-2 (ad541)	DR vs. AL	-	0.0263	-20.89	6.05	-39.61	-2.16
gbp-2 (ad541)	DR vs. AL	+	0.0069	-25.45	6.05	-44.18	-6.73
Influence of the genotype compared to N2							
egl-8 (md1971)	AL	-	<1e-04	-58.06	5.78	-73.93	-42.20
egl-8 (md1971)	AL	+	<1e-04	-59.90	5.69	-75.53	-44.28
egl-8 (md1971)	DR	-	<1e-04	-36.59	5.77	-52.42	-20.77
egl-8 (md1971)	DR	+	0.6900	-9.43	6.39	-26.97	8.11
gbp-2 (ad541)	AL	-	0.2263	-12.02	5.59	-27.35	3.32
gbp-2 (ad541)	AL	+	0.8195	-7.19	5.59	-22.53	8.15
gbp-2 (ad541)	DR	-	0.8602	6.87	5.66	-8.66	22.41
gbp-2 (ad541)	DR	+	0.0026	20.26	5.56	4.99	35.53
Influence of bacteria lawn on plates (+) compared to empty plates (-)							
N2	AL	+ vs. -	0.0683	-21.45	6.51	-44.64	1.74
N2	DR	+ vs. -	0.0085	-34.57	6.56	-57.96	-11.19
egl-8 (md1971)	AL	+ vs. -	0.1105	-23.29	8.05	-51.95	5.38
egl-8 (md1971)	DR	+ vs. -	0.8995	-7.41	8.48	-37.62	22.80
gbp-2 (ad541)	AL	+ vs. -	0.2866	-16.62	7.88	-44.68	11.45
gbp-2 (ad541)	DR	+ vs. -	0.1418	-21.18	7.88	-49.25	6.88

Table S6. Statistical analysis of BBSF increase in response to DR of all mutant strains. Statistics were performed as described in 2.5. by two-way ANOVA and 95 % coverage probability, analysing significant differences between AL and DR condition.

Strain	Estimate (mean difference DR-C)	lower level	upper level
<i>aak-2 (ok524)</i>	17.24	2.68	31.80
<i>aak-2 (rr48)</i>	21.98	7.42	36.54
<i>acr-2 (ok1887)</i>	30.06	6.98	53.15
<i>akt-1 (mg306)</i>	26.67	13.86	39.48
<i>cat-2 (e1112)</i>	33.05	16.85	49.26
<i>che-2 (e1033)</i>	25.45	13.69	37.21
<i>col-179 (ok3010)</i>	24.20	16.13	32.27
<i>col-61 (ok2582)</i>	39.69	17.80	61.57
<i>col-99 (ok1204)</i>	27.63	5.74	49.52
<i>daf-22 (ok693)</i>	18.35	5.69	31.00
<i>daf-7 (e1372)</i>	20.02	6.34	33.69
<i>dgk-1 (nu62)</i>	13.95	5.16	22.74
<i>dpy-5 (e61)</i>	24.53	1.44	47.62
<i>eat-16 (sa609)</i>	31.39	15.12	47.67
<i>eat-2 (ad465)</i>	19.99	7.46	32.51
<i>egl-30 (ad805)</i>	6.67	-15.46	28.81
<i>egl-4 (n478)</i>	39.26	22.70	55.83
<i>egl-8 (e2917)</i>	25.01	12.50	37.52
<i>egl-8 (md1971)</i>	16.01	3.58	28.44
<i>far-4 (ok317)</i>	48.83	25.50	72.16
<i>flp-18 (gk3063)</i>	29.10	7.21	50.99
<i>gbp-2 (ad541)</i>	9.06	1.02	17.09
<i>gbp-2 (pk751)</i>	17.32	3.25	31.39
<i>goa-1 (n1134)</i>	17.06	7.93	26.19
<i>goa-1 (syls9)</i>	0.33	-21.37	22.04
<i>lon-2 (e678)</i>	28.24	14.93	41.55
<i>mec-4 (e1611)</i>	39.06	15.97	62.14
<i>mgl-1 (tm1811)</i>	32.88	19.38	46.38
<i>mgl-2 (tm355)</i>	24.32	12.73	35.91
<i>N2</i>	41.58	36.83	46.34
<i>npr-4 (tm1782)</i>	45.91	29.25	62.57
<i>npr-5 (ok1583)</i>	27.09	5.20	48.98
<i>odr-1 (n1936)</i>	31.39	-1.81	64.58
<i>pept-1 (lg1601)</i>	22.36	6.16	38.56
<i>pgk-10 ::GFP</i>	34.61	15.89	53.34
<i>raga-1 (ok386)</i>	32.27	0.50	64.05
<i>rict-1 (ft7)</i>	37.40	25.97	48.83
<i>rict-1 (mg360)</i>	21.03	8.15	33.91
<i>rsk-1 (ok1255)</i>	35.29	5.81	64.76
<i>ser-2 (pk1357)</i>	40.20	24.82	55.58
<i>ser-3 (ad1774)</i>	10.48	2.56	18.41
<i>sgk-1 (ft15)</i>	21.82	8.88	34.76
<i>snf-1 (ok790)</i>	19.16	2.95	35.36
<i>srp-3 (ok1433)</i>	48.32	25.52	71.11
<i>tph-1 (mg280)</i>	6.69	-23.52	36.90
<i>trp-4 (sy695)</i>	29.10	17.05	41.15
<i>unc-63 (x37)</i>	5.62	-14.75	26.00

Table S7. Statistical analysis of BBSF increase in response to DR of all mutant strains. Statistics were performed as described in 2.5. by two-way ANOVA and 90 % coverage probability, analysing equivalence of AL and DR condition.

Strain	Estimate (mean difference DR-AL)	lower level	upper level
<i>aak-2 (ok524)</i>	17.24	3.77	30.72
<i>aak-2 (rr48)</i>	21.98	8.51	35.45
<i>acr-2 (ok1887)</i>	30.06	8.70	51.43
<i>akt-1 (mg306)</i>	26.67	14.82	38.53
<i>cat-2 (e1112)</i>	33.05	18.06	48.05
<i>che-2 (e1033)</i>	25.45	14.57	36.34
<i>col-179 (ok3010)</i>	24.20	16.73	31.66
<i>col-61 (ok2582)</i>	39.69	19.43	59.94
<i>col-99 (ok1204)</i>	27.63	7.38	47.89
<i>daf-22 (ok693)</i>	18.35	6.63	30.06
<i>daf-7 (e1372)</i>	20.02	7.36	32.67
<i>dgk-1 (nu62)</i>	13.95	5.82	22.08
<i>dpy-5 (e61)</i>	24.53	3.17	45.89
<i>eat-16 (sa609)</i>	31.39	16.33	46.46
<i>eat-2 (ad465)</i>	19.99	8.40	31.57
<i>egl-30 (ad805)</i>	6.67	-13.80	27.15
<i>egl-4 (n478)</i>	39.26	23.93	54.59
<i>egl-8 (e2917)</i>	25.01	13.44	36.59
<i>egl-8 (md1971)</i>	16.01	4.51	27.51
<i>far-4 (ok317)</i>	48.83	27.24	70.42
<i>flp-18 (gk3063)</i>	29.10	8.84	49.36
<i>gbp-2 (ad541)</i>	9.06	1.62	16.49
<i>gbp-2 (pk751)</i>	17.32	4.30	30.34
<i>goa-1 (n1134)</i>	17.06	8.61	25.50
<i>goa-1 (syls9)</i>	0.33	-19.75	20.42
<i>lon-2 (e678)</i>	28.24	15.93	40.56
<i>mec-4 (e1611)</i>	39.06	17.69	60.42
<i>mgl-1 (tm1811)</i>	32.88	20.39	45.37
<i>mgl-2 (tm355)</i>	24.32	13.59	35.04
N2	41.58	37.19	45.98
<i>npr-4 (tm1782)</i>	45.91	30.49	61.33
<i>npr-5 (ok1583)</i>	27.09	6.83	47.34
<i>odr-1 (n1936)</i>	31.39	0.67	62.10
<i>pept-1 (lg1601)</i>	22.36	7.37	37.35
<i>pgk-10 ::GFP</i>	34.61	17.28	51.94
<i>raga-1 (ok386)</i>	32.27	2.87	61.68
<i>rict-1 (ft7)</i>	37.40	26.82	47.98
<i>rict-1 (mg360)</i>	21.03	9.11	32.95
<i>rsk-1 (ok1255)</i>	35.29	8.01	62.56
<i>ser-2 (pk1357)</i>	40.20	25.97	54.43
<i>ser-3 (ad1774)</i>	10.48	3.15	17.82
<i>sgk-1 (ft15)</i>	21.82	9.84	33.79
<i>snf-1 (ok790)</i>	19.16	4.16	34.15
<i>srp-3 (ok1433)</i>	48.32	27.22	69.41
<i>tph-1 (mg280)</i>	6.69	-21.27	34.64
<i>trp-4 (sy695)</i>	29.10	17.95	40.25
<i>unc-63 (x37)</i>	5.62	-13.23	24.48

Table S8. Original data of free amino acid analysis of AL control group.

Results of 3 independent experiments (C1-C3).

<i>Free-amino acids</i>	C1			C2			C3		
	<i>µg/ml</i>	<i>µg/worm</i>	<i>% / worm</i>	<i>µg/ml</i>	<i>µg/worm</i>	<i>% / worm</i>	<i>µg/ml</i>	<i>µg/worm</i>	<i>% / worm</i>
P-Eta	2.2118	0.0004	0.9841	2.4206	0.0005	1.2163	1.7407	0.0003	1.1270
Asp	2.6141	0.0005	1.1631	1.7236	0.0004	0.8661	2.3998	0.0005	1.5537
Thr	6.5958	0.0013	2.9346	4.7847	0.0010	2.4042	3.7974	0.0007	2.4586
Ser	4.5504	0.0009	2.0246	4.1101	0.0009	2.0653	2.5201	0.0005	1.6316
Asn	3.9768	0.0008	1.7694	4.1829	0.0009	2.1019	1.6515	0.0003	1.0692
Glu	20.1524	0.0039	8.9662	22.2622	0.0048	11.1865	14.1304	0.0028	9.1486
Gln	9.4924	0.0018	4.2234	14.8459	0.0032	7.4599	6.4058	0.0013	4.1473
α-AAA	10.9697	0.0021	4.8806	11.7869	0.0026	5.9228	11.1679	0.0022	7.2306
Pro	2.9957	0.0006	1.3328	2.4028	0.0005	1.2074	2.2991	0.0005	1.4886
Gly	3.6326	0.0007	1.6162	2.7558	0.0006	1.3848	2.2784	0.0004	1.4751
Ala	28.5824	0.0055	12.7169	21.9355	0.0048	11.0223	22.0906	0.0043	14.3023
Val	8.7769	0.0017	3.9050	5.0480	0.0011	2.5365	6.7303	0.0013	4.3574
Cys	1.0880	0.0002	0.4841	0.4955	0.0001	0.2490	0.0000	0.0000	0.0000
Cystathionin	8.4959	0.0016	3.7800	9.0687	0.0020	4.5569	9.4172	0.0018	6.0971
Met	4.6822	0.0009	2.0832	3.9705	0.0009	1.9951	2.9887	0.0006	1.9350
Ile	12.9150	0.0025	5.7461	9.7092	0.0021	4.8787	6.6293	0.0013	4.2921
Leu	16.1903	0.0031	7.2034	13.9958	0.0030	7.0327	10.0516	0.0020	6.5078
Tyr	7.3183	0.0014	3.2560	6.3163	0.0014	3.1738	4.4953	0.0009	2.9105
Phe	9.2275	0.0018	4.1055	7.8151	0.0017	3.9270	6.5316	0.0013	4.2288
GABA	2.4831	0.0005	1.1048	0.0000	0.0000	0.0000	2.5698	0.0005	1.6638
His	4.8844	0.0009	2.1732	3.5314	0.0008	1.7745	3.8107	0.0007	2.4672
Trp	2.9450	0.0006	1.3103	3.0492	0.0007	1.5322	0.5085	0.0001	0.3292
Lys	23.2574	0.0044	10.3477	21.5469	0.0047	10.8270	2.9573	0.0006	1.9147
NH3	2.1187	0.0004	0.9427	1.1975	0.0003	0.6017	14.1863	0.0028	9.1848
Arg	22.3725	0.0043	9.9540	20.0556	0.0044	10.0777	13.0964	0.0026	8.4791

Table S9. Original data of free amino acid analysis of DR group.
Results of 3 independent experiments (DR1-DR3).

<i>Free-amino acids</i>	<i>DR1</i>			<i>DR2</i>			<i>DR3</i>		
	<i>µg/ ml</i>	<i>µg/ worm</i>	<i>% / worm</i>	<i>µg/ ml</i>	<i>µg/ worm</i>	<i>% / worm</i>	<i>µg/ ml</i>	<i>µg/ worm</i>	<i>% / worm</i>
P-Eta	1.6518	0.0001	0.9548	2.0256	0.0002	1.0055	1.6518	0.0001	0.9548
Asp	5.3400	0.0004	3.0868	4.7131	0.0004	2.3395	5.3400	0.0004	3.0868
Thr	3.4767	0.0002	2.0097	3.6783	0.0003	1.8258	3.4767	0.0002	2.0097
Ser	3.2094	0.0002	1.8552	4.4926	0.0004	2.2300	3.2094	0.0002	1.8552
Asn	2.5420	0.0002	1.4694	6.1911	0.0005	3.0732	2.5420	0.0002	1.4694
Glu	28.1725	0.0019	16.2850	31.8919	0.0025	15.8306	28.1725	0.0019	16.2850
Gln	9.8564	0.0007	5.6974	19.8326	0.0016	9.8445	9.8564	0.0007	5.6974
α-AAA	10.0637	0.0007	5.8173	7.3894	0.0006	3.6680	10.0637	0.0007	5.8173
Pro	2.5996	0.0002	1.5027	3.1626	0.0003	1.5699	2.5996	0.0002	1.5027
Gly	4.3526	0.0003	2.5160	5.5432	0.0004	2.7515	4.3526	0.0003	2.5160
Ala	34.0540	0.0023	19.6848	33.1327	0.0026	16.4465	34.0540	0.0023	19.6848
Val	3.8495	0.0003	2.2252	3.1396	0.0002	1.5585	3.8495	0.0003	2.2252
Cys	0.0497	0.0000	0.0287	0.7924	0.0001	0.3933	0.0497	0.0000	0.0287
Cystathionin	11.0179	0.0007	6.3688	8.8289	0.0007	4.3825	11.0179	0.0007	6.3688
Met	1.4205	0.0001	0.8211	2.0516	0.0002	1.0184	1.4205	0.0001	0.8211
Ile	3.8735	0.0003	2.2390	4.5280	0.0004	2.2476	3.8735	0.0003	2.2390
Leu	4.5555	0.0003	2.6333	6.7841	0.0005	3.3675	4.5555	0.0003	2.6333
Tyr	2.8682	0.0002	1.6580	0.5581	0.0000	0.2770	2.8682	0.0002	1.6580
Phe	3.6507	0.0002	2.1103	5.6264	0.0004	2.7928	3.6507	0.0002	2.1103
GABA	0.7012	0.0000	0.4053	0.1825	0.0000	0.0906	0.7012	0.0000	0.4053
His	4.5089	0.0003	2.6064	3.9178	0.0003	1.9447	4.5089	0.0003	2.6064
Trp	1.3540	0.0001	0.7827	0.9497	0.0001	0.4714	1.3540	0.0001	0.7827
Lys	1.4714	0.0001	0.8505	16.7534	0.0013	8.3161	1.4714	0.0001	0.8505
NH3	11.1265	0.0007	6.4317	1.8928	0.0001	0.9395	11.1265	0.0007	6.4317
Arg	17.2301	0.0012	9.9598	23.3951	0.0019	11.6129	17.2301	0.0012	9.9598

Table S10. Original data of free amino acid analysis of starved group.

Results of 3 independent experiments (Starv1-Starv3), starvation was performed from AL L4 for 20 h.

<i>Free-amino acids</i>	<i>Starv1</i>			<i>Starv2</i>			<i>Starv3</i>		
	<i>µg/ ml</i>	<i>µg/ worm</i>	<i>% / worm</i>	<i>µg/ ml</i>	<i>µg/ worm</i>	<i>% / worm</i>	<i>µg/ ml</i>	<i>µg/ worm</i>	<i>% / worm</i>
P-Eta	1.3006	0.0001	1.0993	0.9677	0.0001	0.8800	1.0537	0.0001	1.2417
Asp	3.0520	0.0003	2.5796	4.0516	0.0003	3.6844	3.9331	0.0003	4.6348
Thr	2.7969	0.0002	2.3641	1.9951	0.0001	1.8143	1.1152	0.0001	1.3142
Ser	2.4507	0.0002	2.0714	2.4948	0.0002	2.2688	2.2489	0.0002	2.6502
Asn	3.3955	0.0003	2.8700	5.5305	0.0004	5.0294	2.4442	0.0002	2.8803
Glu	21.4339	0.0018	18.1167	21.5310	0.0016	19.5801	19.6713	0.0015	23.1810
Gln	13.1360	0.0011	11.1030	15.0271	0.0011	13.6655	6.4291	0.0005	7.5762
α-AAA	2.6388	0.0002	2.2304	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Pro	0.0000	0.0000	0.0000	1.1778	0.0001	1.0711	0.0000	0.0000	0.0000
Gly	5.1340	0.0004	4.3395	4.0207	0.0003	3.6564	3.6251	0.0003	4.2719
Ala	27.3288	0.0023	23.0992	20.3941	0.0015	18.5462	22.6065	0.0017	26.6398
Val	0.0000	0.0000	0.0000	1.2312	0.0001	1.1197	1.5124	0.0001	1.7822
Cys	0.0000	0.0000	0.0000	0.1890	0.0000	0.1719	0.0000	0.0000	0.0000
Cystathionin	6.1960	0.0005	5.2371	2.7550	0.0002	2.5054	3.4632	0.0003	4.0811
Met	0.4103	0.0000	0.3468	0.7296	0.0001	0.6635	0.0000	0.0000	0.0000
Ile	1.4888	0.0001	1.2584	1.0100	0.0001	0.9185	0.2558	0.0000	0.3014
Leu	2.2115	0.0002	1.8693	2.2325	0.0002	2.0302	0.9707	0.0001	1.1438
Tyr	1.2393	0.0001	1.0475	0.7701	0.0001	0.7003	0.2682	0.0000	0.3160
Phe	2.1722	0.0002	1.8361	2.2069	0.0002	2.0070	1.2868	0.0001	1.5164
GABA	0.2990	0.0000	0.2528	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
His	2.0404	0.0002	1.7246	1.4414	0.0001	1.3108	1.4259	0.0001	1.6803
Trp	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Lys	6.7335	0.0006	5.6914	7.0288	0.0005	6.3919	0.2419	0.0000	0.2851
NH3	1.3678	0.0001	1.1561	1.2728	0.0001	1.1575	4.3448	0.0003	5.1200
Arg	9.2309	0.0008	7.8022	11.9066	0.0009	10.8277	7.9627	0.0006	9.3834

Table S11. Original data of amino acid analysis by total hydrolysis of AL control group.
Results of 3 independent experiments (C1-C3).

<i>total hydrolysis amino acids</i>	C1			C2			C3		
	<i>µg/ml</i>	<i>µg/worm</i>	<i>% / worm</i>	<i>µg/ml</i>	<i>µg/worm</i>	<i>% / worm</i>	<i>µg/ml</i>	<i>µg/worm</i>	<i>% / worm</i>
Asp	172.9861	0.0331	8.8100	220.5374	0.0479	12.9223	187.6790	0.0368	11.2331
Hydroxyprolin	15.4471	0.0030	0.7867	9.0873	0.0020	0.5325	7.8035	0.0015	0.4671
Thr	110.7021	0.0212	5.6380	96.2598	0.0209	5.6403	95.0195	0.0186	5.6872
Ser	77.3494	0.0148	3.9393	67.4058	0.0146	3.9496	67.4426	0.0132	4.0366
Asn	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Glu	209.0423	0.0400	10.6463	207.7740	0.0451	12.1745	210.7005	0.0413	12.6110
Gln	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
α-AAA	11.7273	0.0022	0.5973	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Pro	91.4489	0.0175	4.6574	61.9595	0.0135	3.6305	69.2668	0.0136	4.1458
Gly	85.7299	0.0164	4.3662	102.7543	0.0223	6.0209	98.9543	0.0194	5.9227
Ala	128.4261	0.0246	6.5406	116.0893	0.0252	6.8022	116.0946	0.0228	6.9486
Val	112.0844	0.0214	5.7084	107.5812	0.0234	6.3037	107.7651	0.0211	6.4500
Cys	8.7877	0.0017	0.4476	3.2931	0.0007	0.1930	1.8901	0.0004	0.1131
Cystathionin	11.0800	0.0021	0.5643	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Met	38.7334	0.0074	1.9727	33.1783	0.0072	1.9441	35.7791	0.0070	2.1415
Ile	100.8881	0.0193	5.1381	93.2724	0.0203	5.4653	94.0896	0.0185	5.6315
Leu	144.4300	0.0276	7.3557	134.5083	0.0292	7.8815	133.5757	0.0262	7.9949
Tyr	64.1014	0.0123	3.2646	60.1986	0.0131	3.5273	62.6301	0.0123	3.7486
Phe	85.9979	0.0164	4.3798	78.1084	0.0170	4.5767	80.1651	0.0157	4.7981
GABA	2.9173	0.0006	0.1486	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
His	67.0167	0.0128	3.4131	45.1810	0.0098	2.6474	45.5845	0.0089	2.7284
Trp	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Orn	3.2442	0.0006	0.1652	2.0491	0.0004	0.1201	1.7675	0.0003	0.1058
Lys	235.6115	0.0450	11.9995	136.6570	0.0297	8.0074	133.0300	0.0261	7.9622
NH3	30.3040	0.0058	1.5434	7.5752	0.0016	0.4439	0.0000	0.0000	0.0000
Arg	155.4561	0.0297	7.9172	123.1699	0.0268	7.2171	121.5306	0.0238	7.2739

Table S12. Original data of amino acid analysis by totalhydrolysis of DR group.

Results of 3 independent experiments (DR1-DR3).

<i>total hydrolysis amino acids</i>	<i>DR1</i>			<i>DR2</i>			<i>DR3</i>		
	<i>µg/ ml</i>	<i>µg/ worm</i>	<i>% / worm</i>	<i>µg/ ml</i>	<i>µg/ worm</i>	<i>% / worm</i>	<i>µg/ ml</i>	<i>µg/ worm</i>	<i>% / worm</i>
Asp	142.9348	0.0114	9.5528	254.2143	0.0201	13.0293	253.3598	0.0170	12.0638
Hydroxyprolin	13.7241	0.0011	0.9172	12.2108	0.0010	0.6258	13.1943	0.0009	0.6283
Thr	79.5679	0.0063	5.3178	108.6393	0.0086	5.5681	127.3057	0.0085	6.0617
Ser	60.6727	0.0048	4.0550	76.1125	0.0060	3.9010	90.0821	0.0060	4.2893
Asn	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Glu	181.6158	0.0145	12.1380	258.5368	0.0205	13.2508	291.3821	0.0195	13.8742
Gln	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
α-AAA	7.8037	0.0006	0.5215	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Pro	76.1516	0.0061	5.0895	80.6727	0.0064	4.1347	101.0934	0.0068	4.8136
Gly	70.0839	0.0056	4.6839	121.7185	0.0096	6.2385	134.8385	0.0090	6.4204
Ala	111.5274	0.0089	7.4538	142.4317	0.0113	7.3001	162.8935	0.0109	7.7562
Val	72.0215	0.0057	4.8134	109.1030	0.0086	5.5919	128.0988	0.0086	6.0995
Cys	8.5902	0.0007	0.5741	5.2075	0.0004	0.2669	4.6937	0.0003	0.2235
Cystathionin	9.6903	0.0008	0.6476	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Met	26.7205	0.0021	1.7858	36.6221	0.0029	1.8770	45.5314	0.0030	2.1680
Ile	61.9175	0.0049	4.1382	91.4937	0.0072	4.6893	1.9754	0.0001	0.0941
Leu	102.3664	0.0082	6.8415	143.3229	0.0114	7.3458	165.6625	0.0111	7.8881
Tyr	46.6166	0.0037	3.1155	66.5909	0.0053	3.4130	79.6421	0.0053	3.7922
Phe	63.3851	0.0050	4.2362	87.5127	0.0069	4.4853	102.6276	0.0069	4.8866
GABA	2.3109	0.0002	0.1544	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
His	50.7621	0.0040	3.3926	52.7560	0.0042	2.7039	60.8274	0.0041	2.8963
Trp	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Orn	1.5904	0.0001	0.1063	1.3458	0.0001	0.0690	1.2837	0.0001	0.0611
Lys	165.5982	0.0132	11.0675	153.0127	0.0121	7.8424	172.8565	0.0116	8.2306
NH3	22.3611	0.0018	1.4945	8.7836	0.0007	0.4502	0.0000	0.0000	0.0000
Arg	118.2435	0.0094	7.9026	140.8163	0.0112	7.2173	162.8178	0.0109	7.7526

Table S13. Original data of amino acid analysis by totalhydrolysis of starved group.
Results of 3 independent experiments (Starv1-Starv3), starvation was performed from AL L4 for 20 h.

<i>total hydrolysis amino acids</i>	<i>Starv1</i>			<i>Starv2</i>			<i>Starv3</i>		
	<i>µg/ml</i>	<i>µg/worm</i>	<i>%/worm</i>	<i>µg/ml</i>	<i>µg/worm</i>	<i>%/worm</i>	<i>µg/ml</i>	<i>µg/worm</i>	<i>%/worm</i>
Asp	136.0215	0.0115	9.5700	204.8502	0.0149	15.2722	160.0275	0.0119	13.0965
Hydroxyprolin	9.0755	0.0008	0.6385	8.9234	0.0007	0.6653	8.9877	0.0007	0.7355
Thr	77.3845	0.0065	5.4445	72.7425	0.0053	5.4232	67.1840	0.0050	5.4983
Ser	57.1595	0.0048	4.0215	53.0242	0.0039	3.9531	51.5782	0.0038	4.2211
Asn	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Glu	182.6384	0.0154	12.8498	175.3951	0.0128	13.0762	166.3216	0.0124	13.6116
Gln	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
α-AAA	3.0112	0.0003	0.2119	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Pro	75.7671	0.0064	5.3307	56.3009	0.0041	4.1974	58.1119	0.0043	4.7558
Gly	67.7019	0.0057	4.7633	83.7421	0.0061	6.2432	77.4895	0.0058	6.3417
Ala	104.9233	0.0089	7.3820	98.0831	0.0071	7.3124	96.9800	0.0072	7.9368
Val	68.5070	0.0058	4.8199	71.2623	0.0052	5.3128	65.3873	0.0049	5.3512
Cys	7.5483	0.0006	0.5311	3.2810	0.0002	0.2446	3.3864	0.0003	0.2771
Cystathionin	6.2338	0.0005	0.4386	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Met	24.2839	0.0021	1.7085	23.5319	0.0017	1.7544	22.5516	0.0017	1.8456
Ile	59.1747	0.0050	4.1633	59.1419	0.0043	4.4092	54.3175	0.0040	4.4453
Leu	96.1870	0.0081	6.7674	95.3344	0.0069	7.1075	86.7847	0.0065	7.1024
Tyr	43.1975	0.0036	3.0392	44.4097	0.0032	3.3109	42.4438	0.0032	3.4736
Phe	59.5906	0.0050	4.1926	57.2317	0.0042	4.2668	51.9902	0.0039	4.2548
GABA	2.9100	0.0002	0.2047	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
His	45.1066	0.0038	3.1735	33.7892	0.0025	2.5191	31.3718	0.0023	2.5674
Trp	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Orn	1.5996	0.0001	0.1125	0.7192	0.0001	0.0536	0.4297	0.0000	0.0352
Lys	158.7857	0.0134	11.1716	99.3770	0.0072	7.4089	90.7606	0.0068	7.4278
NH3	22.5111	0.0019	1.5838	6.0646	0.0004	0.4521	0.0000	0.0000	0.0000
Arg	112.0158	0.0095	7.8810	94.1237	0.0069	7.0172	85.8057	0.0064	7.0223

Table S14. Original data of viscosity measurement of CMC dilutions.
Dilutions ranging from 2000 ppm - 10000 ppm.

<i>2000 ppm</i>					
Scherrate [1/s]	Schubspannung [Pa]	Viskosität [mPa·s]	Drehzahl [1/min]	Moment [μNm]	
5	0.04148	8.297	0.8334	1.358	
5.854	0.06001	10.25	0.9757	1.964	
6.854	0.07602	11.09	1.142	2.488	
8.024	0.0658	8.2	1.337	2.153	
9.394	0.0664	7.069	1.566	2.173	
11	0.1054	9.58	1.833	3.448	
12.88	0.1183	9.19	2.146	3.873	
15.08	0.1223	8.109	2.513	4.001	
17.65	0.1727	9.787	2.942	5.653	
20.67	0.1762	8.528	3.445	5.768	
24.19	0.2084	8.612	4.033	6.819	
28.33	0.2655	9.372	4.722	8.688	
33.16	0.3108	9.371	5.528	10.17	
38.83	0.3585	9.233	6.472	11.73	
45.46	0.3932	8.649	7.577	12.87	
53.22	0.4773	8.967	8.871	15.62	
62.31	0.536	8.602	10.39	17.54	
72.95	0.6266	8.589	12.16	20.51	
85.41	0.7547	8.836	14.24	24.7	
100	0.8586	8.586	16.67	28.1	

<i>4000 ppm</i>					
Scherrate [1/s]	Schubspannung [Pa]	Viskosität [mPa·s]	Drehzahl [1/min]	Moment [μNm]	
5.001	0.05173	10.35	0.8335	1.693	
5.853	0.05918	10.11	0.9756	1.937	
6.854	0.08203	11.97	1.142	2.684	
8.024	0.1123	13.99	1.338	3.674	
9.395	0.1208	12.86	1.566	3.953	
11	0.1264	11.49	1.833	4.135	
12.88	0.169	13.12	2.146	5.531	
15.08	0.1908	12.65	2.513	6.243	
17.65	0.2272	12.87	2.942	7.436	
20.67	0.2627	12.71	3.445	8.597	
24.19	0.3185	13.16	4.033	10.42	
28.33	0.3639	12.85	4.722	11.91	
33.16	0.4134	12.46	5.528	13.53	
38.83	0.4909	12.64	6.472	16.07	
45.46	0.5856	12.88	7.577	19.16	
53.22	0.6658	12.51	8.872	21.79	
62.31	0.7931	12.73	10.39	25.95	
72.95	0.926	12.69	12.16	30.3	
85.41	1.065	12.47	14.24	34.84	
100	1.252	12.52	16.67	40.96	

5000 ppm					
Scherrate [1/s]	Schubspannung [Pa]	Viskosität [mPa·s]	Drehzahl [1/min]	Moment [μNm]	
5	0.1144	22.89	0.8334	3.745	
5.854	0.1405	23.99	0.9758	4.596	
6.854	0.155	22.62	1.142	5.073	
8.025	0.1613	20.09	1.338	5.277	
9.395	0.1953	20.79	1.566	6.393	
11	0.253	23	1.833	8.278	
12.88	0.2697	20.94	2.147	8.825	
15.08	0.328	21.76	2.513	10.74	
17.65	0.3861	21.88	2.942	12.64	
20.67	0.4534	21.94	3.445	14.84	
24.2	0.5103	21.09	4.033	16.7	
28.33	0.6123	21.62	4.722	20.04	
33.16	0.7216	21.76	5.528	23.61	
38.83	0.8356	21.52	6.472	27.35	
45.46	0.9592	21.1	7.577	31.39	
53.22	1.148	21.58	8.871	37.58	
62.31	1.318	21.16	10.39	43.14	
72.95	1.536	21.06	12.16	50.27	
85.41	1.803	21.1	14.24	58.99	
100	2.088	20.88	16.67	68.33	

6000 ppm					
Scherrate [1/s]	Schubspannung [Pa]	Viskosität [mPa·s]	Drehzahl [1/min]	Moment [μNm]	
5	0.3653	73.06	0.8334	11.95	
5.854	0.4227	72.21	0.9757	13.83	
6.853	0.5001	72.97	1.142	16.36	
8.024	0.5985	74.6	1.337	19.59	
9.395	0.6887	73.31	1.566	22.54	
11	0.7847	71.34	1.833	25.68	
12.88	0.9279	72.06	2.146	30.37	
15.08	1.076	71.39	2.513	35.22	
17.65	1.243	70.39	2.942	40.66	
20.67	1.439	69.63	3.445	47.09	
24.2	1.68	69.44	4.033	54.98	
28.33	1.922	67.86	4.722	62.9	
33.17	2.204	66.46	5.528	72.13	
38.83	2.538	65.37	6.472	83.06	
45.46	2.926	64.37	7.577	95.77	
53.22	3.32	62.39	8.871	108.7	
62.31	3.813	61.19	10.39	124.8	
72.95	4.341	59.51	12.16	142.1	
85.41	4.918	57.58	14.24	161	
100	5.592	55.92	16.67	183	

7000 ppm					
Scherrate [1/s]	Schubspannung [Pa]	Viskosität [mPa·s]	Drehzahl [1/min]	Moment [μNm]	
5	0.2223	44.46	0.8334	7.275	
5.854	0.2586	44.18	0.9758	8.464	
6.854	0.2883	42.06	1.142	9.433	
8.024	0.3182	39.65	1.338	10.41	
9.394	0.3849	40.97	1.566	12.59	
11	0.466	42.36	1.833	15.25	
12.88	0.5212	40.47	2.146	17.06	
15.08	0.6225	41.29	2.513	20.37	
17.65	0.7215	40.87	2.942	23.61	
20.67	0.8544	41.34	3.445	27.96	
24.2	0.9755	40.32	4.033	31.92	
28.33	1.149	40.56	4.722	37.6	
33.16	1.349	40.68	5.528	44.15	
38.83	1.566	40.33	6.472	51.25	
45.46	1.813	39.88	7.577	59.33	
53.22	2.133	40.08	8.871	69.81	
62.31	2.462	39.51	10.39	80.56	
72.95	2.856	39.15	12.16	93.46	
85.41	3.326	38.94	14.24	108.9	
100	3.847	38.47	16.67	125.9	

8000 ppm					
Scherrate [1/s]	Schubspannung [Pa]	Viskosität [mPa·s]	Drehzahl [1/min]	Moment [μNm]	
5	0.2942	58.85	0.8334	9.629	
5.854	0.3563	60.87	0.9758	11.66	
6.853	0.4251	62.03	1.142	13.91	
8.024	0.4901	61.08	1.337	16.04	
9.394	0.551	58.66	1.566	18.03	
11	0.6667	60.61	1.833	21.82	
12.88	0.7872	61.13	2.146	25.76	
15.08	0.8932	59.24	2.513	29.23	
17.65	1.072	60.7	2.942	35.06	
20.67	1.225	59.29	3.445	40.1	
24.2	1.442	59.58	4.033	47.17	
28.33	1.687	59.56	4.722	55.21	
33.16	1.961	59.12	5.528	64.17	
38.83	2.278	58.66	6.472	74.54	
45.46	2.623	57.69	7.577	85.83	
53.22	3.044	57.2	8.871	99.62	
62.31	3.515	56.4	10.39	115	
72.95	4.061	55.67	12.16	132.9	
85.41	4.703	55.07	14.24	153.9	
100	5.41	54.1	16.67	177.1	

<i>10000ppm</i>					
Scherrate [1/s]	Schubspannung [Pa]	Viskosität [mPa·s]	Drehzahl [1/min]	Moment [μNm]	
5	1.675	334.9	0.8334	54.81	
5.854	1.906	325.5	0.9757	62.36	
6.856	2.19	319.4	1.143	71.65	
8.024	2.519	313.9	1.338	82.44	
9.394	2.895	308.1	1.566	94.73	
11	3.299	299.9	1.833	108	
12.88	3.789	294.2	2.146	124	
15.08	4.334	287.5	2.513	141.8	
17.65	4.906	277.9	2.942	160.5	
20.67	5.575	269.8	3.445	182.4	
24.19	6.344	262.2	4.033	207.6	
28.33	7.138	252	4.722	233.6	
33.16	8.037	242.3	5.528	263	
38.83	9.036	232.7	6.472	295.7	
45.46	10.17	223.7	7.577	332.8	
53.22	11.32	212.7	8.872	370.5	
62.31	12.69	203.6	10.39	415.2	
72.96	14.12	193.6	12.16	462.2	
85.41	15.6	182.7	14.24	510.6	
100	17.34	173.4	16.67	567.4	

Table S15. Short Description of used mutant strains. Descriptions were taken from WormBase (Version WS234), 2012. www.wormbase.org

<i>strain</i>	<i>short description</i>
N2 (Bristol)	<i>C.elegans</i> wild isolate
aak-2	AMP-activated Kinase; encodes <i>C.elegans</i> homologs of catalytic alpha subunits of AMP-activated protein kinase; functions downstream of environmental stressors, energy level signals (AMP:ATP ratio) and daf-2-mediated insulin signalling
acr-2	AcetylCholine Receptor; Homolog of non-alpha nicotinic acetylcholine receptor subunit
akt-1	AKT kinase family, ortholog of serine/threonine kinase Akt/PKB; genetically interacts with insulin signalling pathway
cat-2	abnormal CATecholamine distribution; encodes a tyrosine hydroxylase; rate of limiting enzyme in the synthesis of catecholamines (e.g. dopamine)
che-2	abnormal CHEmotaxis; encodes a protein containing G-Protein beta-like WD-40 repeats
col-179, col-61, col-99	COLlagen
daf-22	abnormal DAuer Formation; encodes ortholog of human sterol carrier protein SCP2, which catalyzes the final step in peroxisomal fatty acid beta oxidation; required for dauer pheromone biosynthesis
daf-7	abnormal DAuer Formation; encodes a member of the transforming growth factor superfamily
dgk-1	DiacylGlycerol Kinase; ortholog of mammalian diacylglycerol kinase theta; functions downstream in a serotonin signalling pathway that regulates locomotion and synaptic transmission
dpy-5	DumPY:shorter than wild type; encodes a cuticle procollagen, required for body length
eat-16	EATing: abnormal pharyngeal pumping; encodes an RGS protein that affects movement, pharyngeal pumping, egg laying and synaptic transmission
eat-2	EATing: abnormal pharyngeal pumping; ecodes a ligand-gated ion channel subunit, related to non-alpha-subunits of nicotinic acetylcholine receptors; functions postsynaptically in pharyngeal muscle to regulate the rate of pharyngeal pumping
egl-30	EGg Laying defective; ortholog of the heterometric G protein alpha subunit Gq that affects viability, locomotion, egg laying, synaptic transmission and pharyngeal pumping
egl-4	EGg Laying defective; encodes a cyclic GMP-dependent protein kinase that may act through the TGF-beta signalling pathway
egl-8	EGg Laying defective; encodes a phospholipase C beta homolog that affects pharyngeal pumping, defecation, and activity levels; genetically downstream of <i>egl-30</i>
far-4	Fatty Acid/Retinol binding protein; Encodes a fatty acid and retinol-binding protein
flp-18	FMRF-Like Peptide; encodes the only FMRFamide related neuropeptide (FaPP) encoded by the <i>C.elegans</i> genome
gbp-2	G Protein, Beta subunit; encodes an ortholog of Gbeta(5), that is dispensable for viability, but required for normal egg-laying, locomotion and pharyngeal pumping; GBP-2 regulates the interaction between GOA-1 and EGL-30 signalling pathways
goa-1	G protein, O, Alpha subunit; ortholog of heterotrimeric G protein alpha subunit Go; GOA-1 activity is required for regulation of a number of behaviours, i.e. locomotion, egg-laying, male mating, and olfactory-mediated behaviours; goa-1 genetically interacts with egl-30 pathway
lon-2	LONG; encodes a member of glypican family of heparan sulfate proteoglycans; regulates body length

<i>strain</i>	<i>short description</i>
<i>mec-4</i>	MEChanosensory abnormality; encodes an amiloride-sensitive Na ⁺ channel protein required to sense gentle mechanical stimuli along the body wall
<i>mgl-1</i>	Metabotropic GLutamate receptor family; encodes an group II metabotropic glutamate receptor; predicted to function as a pre-synaptic G-protein coupled receptor
<i>npr-5</i>	NeuroPeptide Receptor family; encodes by alternative splicing, two isoforms of a receptor activated by FLP-18
<i>odr-1</i>	ODoRant response abnormal; encodes a putative guanylyl cyclase, odr-1 activity is required for normal responses to all AWC-sensed odorants
<i>ser-3</i>	SERotonin/octopamine receptor family; encodes an octopamine receptor required in SIA neurons for response in absence of food, ser-3 is also required for normal inhibition of movement by 5-HT
<i>sgk-1</i>	Serum- and Glucocorticoid- inducible Kinase homolog; encodes an serine/threonine protein kinase
<i>snf-1</i>	Sodium: Neurotransporter symporter Family
<i>srp-3</i>	SeRPin; encodes a functional serpin (serine protease inhibitor)
<i>tph-1</i>	TryPtophan Hydroxylase; encodes enzyme catalyzing the rate-limiting first step in serotonin biosynthesis
<i>trp-4</i>	TRP (transient receptor potential) channel family; encodes a pore-forming subunit of a mechanosensitive TRPN channel
<i>unc-63</i>	UNCoordinated; encodes an alpha subunit of a levamisole-sensitive nicotinic acetylcholine receptor, required for normal locomotion

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Eidesstattliche Erklärung

Hiermit versichere ich, daß ich die vorliegende Arbeit, abgesehen von der wissenschaftlichen Beratung meines Betreuers, selbstständig und nur unter Verwendung der hierin angegebenen Quellen und Hilfsmitteln angefertigt habe.

Diese Arbeit wurde, weder in Teilen noch vollständig, an anderer Stelle im Rahmen eines Prüfungsverfahrens vorgelegt.

Des Weiteren ist diese Arbeit unter Einhaltung der Regeln guter wissenschaftlicher Praxis der Deutschen Forschungsgemeinschaft entstanden.

Kiel, 18.12.12

Ulla Faust

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Caricature from Punch's Almanach, 1882