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Use of *eat-5* as a selection marker for transgenics

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eat-5 encodes a *Caenorhabditis elegans* innexin, a component of gap junctions between the muscle cells in the pharynx. The *eat-5* null worms grow normally on an easy-to-eat strain of *E. coli*, HB101, but arrest as L1s on a harder-to-eat strain, DA837, a derivative of OP50. This difference in growth on two *E. coli* strains makes *eat-5* a convenient co-transformation marker. This is particularly useful for situations when *rol-6* is not good because the movement phenotype is studied. The gene of interest is co-injected into the *eat-5(ad1402)* null strain DA1402 with the genomic fragment containing the *eat-5* gene, and the injected worms are transferred on the DA837 *E. coli*. Only transgenic F1s will grow. (It is important to use the null allele *ad1402*--mutants carrying the weak allele *ad464* can grow on DA837.) Once the transgenic strains are isolated, they can be maintained on DA837, so a population of adult transgenic worms is easily obtained. If non-transgenic sibs are needed for controls, a transgenic worm can be transferred on HB101, so that non-transgenics can grow. In this case, however, a second marker, such as GFP, is needed to identify transgenics.

We tested this idea by co-injecting three constructs into DA1402: a constitutively active *glr-1* receptor under its own promoter, *glr1::glr1(Ala->Thr)*, which is known to cause an increased reversal frequency phenotype, *myo2::GFP* (pPD118.33), to have an additional label for the transgenics, and a genomic *eat5::eat5* fragment generated by PCR. Several dozens transgenic lines were established on DA837. Because all adult worms on DA837 are transgenics, it was very easy to see the increased reversal frequency phenotype by looking at the population of worms.

A method of growing large synchronous population of C.elegans using chicken eggs.

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It is sometimes required to prepare a large synchronously growing population of worms for various biochemical or genetic assays. Use of chicken eggs to grow gram quantity of worms has been described (D.Baillie and R.Rosenbluth; WBG2(1)). We have found that by adding E.coli to blended chicken egg it is possible to grow a large population of worms synchronously on plates. In this method, freshly hatched L1 larvae are suspended in blended chicken egg solution supplemented with concentrated E.coli, and seeded onto standard NGM plates. Typically, 1 million worms can be grown synchronously on five, 150mm NGM plates. 1) Take one egg and break into 25ml of boiling water placed on magnetic stirrer. 2) Stir several minutes until yolk breaks. 3) Put the egg solution into Waring blender and blend for two minutes. 4) Transfer the homogenized egg solution to sterile tubes. (They can be frozen and stored at -80C.) 5) Mix egg solution and concentrated E.coli solution (OP-50 grown in Terrific Broth for one day is pelleted and then resuspended in equal volume of sterile M9). Usually we use the ratio of 1:9 = concentrated E.coli : egg solution. 6) Suspend 200,000 freshly hatched L1 larvae in 1~1.5ml of egg/E.coli mixture and inoculate onto 150mm NGM plates.

Stanford males are still the greatest!

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In previous investigations of male fertility in different wild isolates of *C. elegans*, it was found that males from the Stanford race CB4855 are substantially more fertile than males from the standard Bristol race N2 (Hodgkin and Doniach (1997), Genetics 146: 149-164). More recently, several investigators have observed that the Hawaiian race CB4856 also exhibits high male fertility, so that male plates can be maintained indefinitely, simply by chunking rather than by setting up new crosses periodically (Hammarlund & Jorgensen (2001), wbg17.1p38).

We were curious as to whether high male fertility occurred in any other wild isolates. We therefore tested 6 - 12 individual males from a set of 11 geographically assorted races for the ability to sire progeny after more than three days of adult growth at 20 degrees C. Males were tested by daily transfer to fresh plates containing six young adult *fem-1(hc17*ts) females, as described (Hodgkin and Doniach, 1997). The races examined were: N2 (Bri), CB4855 (Sta), CB4856 (Haw), AB1 (Ado), AB3 (Adt), RC301 (Fre), CB4932 (Tau), CB4857 (Cla), CB4854 (Gan), CB4853 (Gat), DH424 (Epc).

The table below reports the fractions of males capable of siring progeny on days 3 - 10 of adulthood. We confirmed long-term fertility for CB4855 (Sta) and CB4856 (Haw). The only other race in this set that exhibited comparable endurance was the Adelelaide race AB1 (Ado).

Rough estimates of total progeny sired at late time points suggest that CB4855 (Sta) has the highest male fertility of these three races, and AB1 (Ado) the lowest.

Other races appear similar to or slightly more fertile than N2 in this survey, which is admittedly superficial. The Californian Epc (DH424) race appears less fertile, perhaps because this strain carries a high Tc1 transposon load. Very high Tc1 loads, as in the Bergerac strains CB4851 and RW7000, are associated with effectively complete male infertility. The Vancouver race KR314 also exhibits male infertility, as a result of homozygosity for a *mab-23* mutation. Overall, we tentatively conclude that high male fertility is the exception rather than the rule, among different wild populations of *C. elegans.*

Day	3	4	5	6	7	8	9	10
Strain								
Bri	10/10	3/10	0/10					
Sta	10/10	10/10	9/10	8/10	8/10	3/10	1/10	0/10
Haw	10/10	7/10	4/10	3/10	3/10	2/10	0/10	
Ado	12/12	12/12	11/12	8/12	6/12	3/12	1/12	0/12
Adt	10/10	5/10	0/10					
Fre	10/10	2/10	1/10	1/10	0/10			

Tau	10/10	3/10	0/10				
Cla	6/6	6/6	3/6	1/6	1/6	0/6	
Gan	6/6	5/6	3/6	2/6	1/6	0/6	
Gat	4/6	3/6	2/6	1/6	1/6	1/6	0/6
Epc	6/12	2/12	0/12				

Biased asymmetry of the RID motor axon

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The nervous system of *C.elegans* displays significant patterns of bilateral symmetry (Hobert et al., 2002). Deviations from this symmetry are usually highly stereotyped. For example, the unilateral RIS neuron is always located on the left side of the animal, but never on the right side (White et al., 1986; this observation, based on a limited number of EM-reconstructed animals, has now been confirmed with *gfp* reporters). One major exception to this stereotyped laterality is represented by the expression of the putative odorant receptor gene *str-2,* which occurs stochastically in either the left or right AWC neuron (the distribution is about 50:50; Troemel, et al., 1999). Are there other examples of stochastic laterality in the nervous system? In their EM reconstruction work, White et al. (1986) noted that the unilateral RID motor neuron (see Fig.), whose cell body is located on the dorsal midline, sends its axon along the right side of the nerve ring in one reconstructed animal, but along the left side in another reconstructed animal. Could this observation reflect a stochastic choice of the growth cone when encountering the dorsal side of the nerve ring? The sample size of two obviously did not allow one to derive such a statement, but the advent of *gfp* reporter technology allows us to address this question. Several previously described *gfp* reporter strains show RID expression, yet the presence of GFP in the axons of many other neurons precluded the tracing of the RID axon. A promoter fragment from a putative serotonin-like receptor gene that we study in the lab drives expression of *gfp* in RID and only one additional pair of neurons, allowing us to trace the axon of RID. Taking advantage of this, we found that in 56 out of 58 animals, the axon traveled along the left side of the nerve ring, while in 2/58 animals, the axon migrated on the right side. In another genetic background in which a *gfp* marker is ectopically expressed in RID (*Is[unc-119::ttx-3; ttx-3::gfp]*), we found a similar distribution; namely 46 out of 48 animals have their RID axon on the left side.

Another case of strongly biased, though not 100% stereotyped left/right asymmery can be found in the ventral nerve cord. The total set of D-type motorneurons sends 2 circumferential commissures around the left side of the animal; the remaining commissures are around the right side of the animal. However, this choice is not entirely stereotyped. In 25% (n=80) of wild type animals there are one or two additional commissures that run on the left side of the animal rather than on the right side.

Taken together, the nervous system of *C.elegans* shows several examples of non-stereotyped, biased anatomic asymmetries. It will be interesting to investigate the mechanistic basis for this left/right asymmetry.

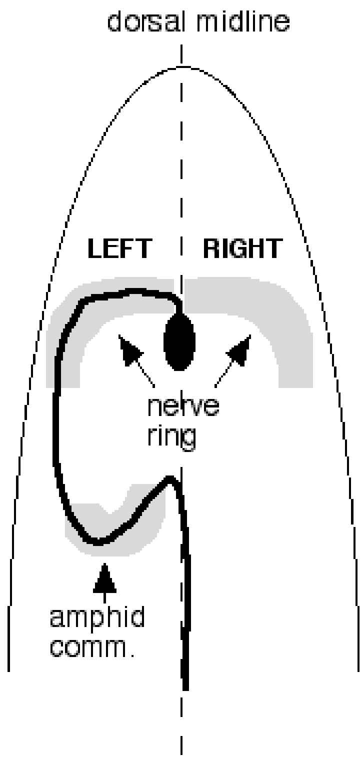

Figure: Morphology of the RID motorneuron.

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Array formation and integration after injection of DNA into oocyte nuclei.

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We are exploring methods for gene targeting. Such methods require the introduction of linear DNA fragments which can then be integrated into chromosomal DNA by means of homologous recombination. However, injection of linear fragments into the gonadal syncytium does not result in homologous recombination of the gene fragment and the chromosome. Rather, such DNA is assembled into extrachromosomal arrays. These arrays are most likely formed using the nonhomologous end joining pathway. We think that it is likely that the nonhomologous end joining happens in the cytoplasm, before the DNA has access to recombine with the chromosome. Thus, this pathway is in direct competition with the homologous recombination pathway that we hope to use in gene targeting. In order to achieve gene targeting we have been working to both increase the frequency of homologous recombination and decrease the frequency of nonhomologous end joining. In all cases, we injected a rescuing fragment from the *unc-18* gene into the *unc-18(e234)* strain and scored for rescued animals. DNA was injected directly into the nucleus of 5-10 oocytes per worm at a concentration of ~100ng/ul. The DNA could be incorporated in one of three ways: end joining into semi-stable arrays, integrating randomly into chromosomal DNA, or homologous recombination into the *unc-18* locus. Transformed animals were tested for integration by isolating true-breeding rescued lines. Homologous integrations were tested by outcrossing and testing for linkage.

First, it is possible that DNA injected into the gonadal syncytium does not enter the nucleus efficiently or perhaps it does not enter at all until after nuclear envelope breakdown. By contrast, injection of circular DNA into the nucleus has resulted in frequent nonhomologous integrations and rare homologous integrations (Broverman, MacMorris, and Blumenthal PNAS 90: 4359-4363 1993). We injected circular DNA into the nucleus but did not identify homologous integrants (see Table); however our methods were unable to generate nonhomologous integrants at high frequencies.

Second, linear DNA with 3’ overhangs may inhibit nonhomologous end-joining as well act as a substrate for RAD-51 binding to initiate strand invasion with homologous sequences. Digestion of 5’ ends was performed by 15 minute treatment with lambda exonuclease at 1unit/ug DNA at 15ºC, leaving 3’ overhangs that were estimated to be 500 nucleotides on each end. DNA treated in this way did not generate homologous integrations.

Third, it is possible that strand invasion by 3’ single stranded overhanging ends can be promoted by prebinding the DNA with RecA protein. RecA is a bacterial homolog of Rad51, which is the protein that binds to 3’ overhangs and catalyzes the integration of single stranded DNA into homologous double-stranded DNA. It was also thought that RecA bound to the free ends of the injected DNA would help protect the DNA from end joining. We did not identify any homologous events. The numbers of arrays and random integrants are proportionally similar with or without RecA.

Although none of our events is a homologous targeting event, our nonhomologous integrations suggest that it is true that a major barrier to homologous integration is access to the nucleus. It has been observed in a number of systems that the majority of integration events are nonhomologous, suggesting that homologous targeting in worms will require a method that generates a large number of integration events.

DNA	injected animals	transformants	integrations	homologous recombinants
Circular (vit-2 Broverman, MacMorris, and Blumenthal 1993)			63	2
Circular	102	42	0	0
Linear	322	119	4	0
Linear + RecA protein	176	78	2	0

A Method for the Cryo-Preservation of *Acrobeloides* (Cephalobidae)

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Introduction:

Preservation of nematode strains at low temperature is widely used. There is a general understanding that the methods hitherto developed are more applicable to Rhabditida, but less to Cephalobida. Consequently cephalobids have to be maintained in culture for future use. Maintaining cultures is time consuming and labour intensive.

We have over 100 culture isolates of *Acrobeloides* which we have been maintaining for the last three years. The objective of this experiment on methodology was, therefore, to be able cryo-preserve these culture isolates. All strains were maintained on *E. coli* (strain OP50) seeded MYOB plates. The standard freezing protocol for *C. elegans* was first tested (Sulston and Hodgkin, 1988), but the method did not work for our isolates. On the other hand culture isolates of a different genus, *Panagrolaimus,* were frozen successfully following the *C. elegans* protocol. Recovery was high (80-90%) for *Panagrolaimus*. We subsequently developed a method of freezing Acrobeloides sp which may be of utility for other nematode zoo-keepers.

Description of the method for the freezing of *Acrobeloides*:

Solutions needed for cryo-preservation are *M9 buffer* and *Freezing solution,* as described for *C. elegans* in Sulston and Hodgkin (1988).

1. Transfer adults to newly seeded plates and maintain cultures at room temperature for ten days until growth is high and peak density is reached. Make sure the density of <u>adults</u> in the culture is high, as they survive freezing better than juveniles (unlike the *C. elegans* protocol that works mainly for larvae).
2. Keep cultures at 15°C for about 15 days. This slows down their metabolism and may starve them to a certain extent.
3. Wash nematodes with *M9 Buffer* from plates into 15ml plastic centrifuge tube and keep them at 4°C for three to four days. This starves them further.
4. Centrifuge at 2000RPM for three minutes. If the culture is dominated by adults, they tend to settle fast and a shorter centrifugation time can be used.
5. Pipette off supernatant leaving about 0.5ml (with nematodes) at the bottom.
6. Heat-shock them by leaving them at 37°C for 45 minutes.
7. Add 1ml freezing solution, mix gently but well and transfer into 1.5ml freezing vials, close and immediately keep them cool in ice. These nematodes seem not to like the freezing solution; therefore the speed of cooling and freezing are critical. The longer one leaves nematodes in the freezing solution, the fewer nematodes will recover from freezing.
8. Put them in a Styrofoam (closed on the bottom but open at the top) and freeze them (at -75°C). Fast thawing is best for survival of nematodes. Keep vials at room temperature but hold them by hand to facilitate thawing. Shake them and pour out into a petri dish half-filled with *M9 Buffer* before they defrost completely in the vial. Slow thawing (first keeping frozen vials at 4°C until they defrost) decreases the recovery of *Acrobeloides* strains we tested.

Normally some nematodes start to show signs of movement about three hours from thawing, but noticeable body movement can be seen much later, usually 24 hours after thawing. Nematodes can be transferred to seeded plates after washing them in buffer. Recovered nematodes transferred to seeded plates reproduced well in culture.

Additional observations:

1. Fast growing cultures of *Acrobeloides* do not survive freezing as well as aged ones.

2. Survival of larvae is much lower than adults. In most cases larvae die while adults survive.

3. The "Slow-Freezing" protocol described for *C. elegans* (Carmel L., web-published) does not work for *Acrobeloides*. Slow freezing according to the *C. elegans*-protocol requires keeping nematodes in *Freezing Solution* and *Buffer* at low temperature before freezing, but this results in the death of all stages of *Acrobeloides*.

4. Exposure of nematodes to heat (37°C) for longer than 45 minutes decreased recovery. But we did not investigate the effect of different levels of temperature on recovery.

5. Total density is not an indicator of degree of recovery, the number of starved adults is.

6. In all cases nematodes were frozen for 24hrs only. The effect of long term freezing was not investigated.

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Evolution of sex differences in lifespan in nematodes

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N2 males live ~20% longer than hermaphrodites when maintained in isolation to prevent deleterious interactions with other worms (1). We have found that this is also true of 9/12 other *C. elegans* wild isolates tested. Increased male lifespan is therefore typical of *C. elegans* as a species, and not unique to N2. Why might this male longevity bias have evolved? One possibility is that it is a consequence of protandrous hermaphroditism, which is likely to lead to a skew in the male reproductive probability distribution to later ages. The evolutionary theory of aging suggests that this would result in the evolution of greater longevity in males. To test this idea, we measured the lifespans of both sexes of four dioecious and three more hermaphroditic terrestrial nematode species. However, males proved to be significantly longer-lived than hermaphrodites/females in all other nematode species tested, bar one. This disproved our hypothesis, and suggested that a male longevity bias is typical of free-living nematodes. The exception was *C. briggsae*, since hermaphrodites were significantly longer-lived than males in the three isolates tested, G16, HK104 and VT847. This could be an evolutionary consequence of the fact that following mating in this species, oocytes are preferentially fertilised by X-bearing sperm, so that outcross progeny are initially mainly hermaphrodites (2). Given that the frequency of males among progeny of selfing hermaphrodites is comparable to that in *C. elegans* (3), it seems likely that *C. briggsae* males are exceptionally rare in the wild. Potentially, this rarity results in reduced selection against deleterious mutations with male-specific effects, and the evolution of reduced male longevity. Interestingly, lifespans of males of dioecious species as a whole were greater than those of males of hermaphroditic species ($p < 0.001$); females were not longer-lived overall than hermaphrodites. Thus, the rarity of males in hermaphroditic species may lead to the evolution of reduced male lifespan.

Male *C. elegans* are also more likely to form dauer larvae than hermaphrodites (4). We have found that insulin/IGF-like signalling plays a role in both increased male dauer formation and lifespan in this species. Possibly increased male lifespan evolved due to selection for increased dauer formation. Yet in other species tested we saw increased dauer formation in hermaphrodites/females, arguing against this idea. However, since this included *C. briggsae*, where hermaphrodites are the longer-lived sex, it is possible that common mechanisms underlie the evolution of sex differences in dauer formation and lifespan in the genus *Caenorhabditis*.

Why might male free-living nematodes of both hermaphroditic and dioecious species be longer-lived than the feminine sex? Within a species, the sex experiencing greater extrinsic mortality is expected to become the one with more rapid aging (5). One cause of early mortality in *C. elegans* hermaphrodites is internal hatching of eggs (bagging). If bagging occurs at significant rates in terrestrial nematodes, the resulting bias in sex-specific survival could account for the evolution of the male longevity bias. Using a simple starvation test, we observed varying levels of bagging in six other terrestrial nematodes tested. In conclusion, a male longevity bias is common among dioecious and hermaphroditic terrestrial nematode species, and may have evolved as a consequence of bagging, while reduced male longevity in hermaphroditic relative to dioecious species may have evolved as a consequence of male rarity.

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The CAST and RIM/UNC-10 system in *C. elegans*

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The cytomatrix at the active zone (CAZ) has been implicated in defining the site of Ca^{2+}-dependent exocytosis of neurotransmitter. Recently, we have identified a novel CAZ protein of ~120 kDa from rat brain and named it CAST (CAZ-associated structural protein) (1). CAST has no transmembrane segment, but has four coiled-coil domains and a C-terminal consensus motif for binding to PDZ domains (2). CAST is localized at the CAZ of conventional synapses of mouse brain. CAST binds directly RIM1 and indirectly Munc13-1 presumably through RIM1, forming a ternary complex. Our cell biological analyses in primary cultured rat hippocampal neurons demonstrate that CAST plays a role at least partly in the localization of RIM1 at the CAZ.

In *C. elegans*, UNC-10 has been identified and characterized as an orthologue of RIM1 (3). In the mutant animals, vesicle priming was impaired, but the organization of the active zone was intact. With GenBank database search, we then identified a putative orthologue of CAST (CeCAST) (F42A6.9: GenBank accession no. AF038613) in *C.elegans*. CeCAST consists of 836 amino acids (aa) with several coiled-coil regions. CeCAST shows a relatively low homology to CAST over the entire sequence (~20% aa identity), but the C-terminal consensus motif of CAST was also conserved in CeCAST. Because this motif was essential for CAST binding to RIM1 in the mammalian system (1), we examined whether CeCAST binds RIM/UNC10. We constructed two CeCAST mutants, pCMV-Myc-CeCAST-1 (residues 581-836) and pCMV-Myc-CeCAST-1deltaC (residues 581-833). The extract of HEK293 cells expressing each mutant was incubated with glutathione Sepharose beads containing a GST fusion protein of RIM/UNC-10 PDZ domain. Myc-CeCAST-1 bound to the beads, but Myc-CeCAST-1deltaC did not. This result suggests that CeCAST directly binds RIM/UNC-10 and that the CAST and RIM/UNC-10 system is also conserved in *C. elegans*. Cell biological and genetic analyses of CeCAST are currently under way.

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Plague? Bah! Mutants resistant to the bubonic plague biofilm

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Bubonic plague is spread by the bites of fleas infected with *Yersinia pestis* bacteria. When worms are exposed to *Y. pestis,* sticky biofilms appear on their heads. The biofilms cover mouths and impair feeding, which in the case of larvae delays or arrests growth. Bacterial genes required for flea infection are also required to form worm biofilms, suggesting that the plague-flea interaction can be modeled with *C. elegans* (1).

N2 worms were mutagenized with ENU and the F2 eggs were deposited on lawns of *Y. pseudotuberculosis,* a close relative of the plague bacillus that also produces biofilms. We screened for animals that had no biofilm and grew normally in the continuous presence of *Yersinia*, a phenotype we call Bah: Biofilm Absent on Head. We obtained 11 independent Bah mutants from 7,000 mutagenized genomes, a frequency that suggests mutations in multiple genes. There are no obvious secondary phenotypes in the mutants.

Biofilms appear on the exterior of worms, and *Yersinia* does not colonize interior tissues, suggesting that some Bah mutants will have altered surface characteristics. Furthermore, three mutations affecting the worm surface -- *srf-2, srf-3,* and *srf-5* -- have been shown to confer resistance to adherent infection by the nematode pathogen *Microbacterium nematophilum* (2). We found that animals with the same three *srf* mutations are Bah. Because the bacterial genera *Microbacterium* and *Yersinia* are not closely related, this commonality suggests that *srf-2, srf-3* and *srf-5* are important in a variety of interactions between nematodes and bacteria.

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C.elegans as a model to study human diseases linked to the mitochondrial respiratory chain complex II

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The mitochondria play a critical role in the cellular energy supply and perform other essential roles in a variety of metabolic functions. The mitochondria electron transport respiratory chain (MRC) is typically composed of four multi-subunit enzymes (complexes I to IV) and the ATP synthase (complex V). Complex II is involved in both the tricarboxylic acid (TCA) cycle and the aerobic respiratory chains of mitochondria. It consists of 4 nuclear-encoded polypeptides: the flavoprotein (Fp/ SDHA), an iron-sulphur protein (Ip/ SDHB) and two integral membrane proteins (SDHC and SDHD). Clinically, complex II deficiency associated with SDHA gene mutations can result in myopathy, encephalopathy and isolated cardiomyopathy. Recently, the analysis of the susceptibility gene for familial paraganglioma syndrome revealed germ line mutations in the SDHB, SDHC and SDHD genes. Those genes are therefore considered to be tumor-suppressor genes. One can speculate that oxidative damage or ROS recycling dysfunction from impaired complex II could be important in explaining the damage to muscle and nerve cells. The molecular basis of such MRC enzyme deficiencies in humans remains largely unknown (1).

We aim to generate *C. elegans* models of these pathologies to identify the underlying mechanisms associated with such deficiencies. We identified by sequence comparison *C. elegans* homologues of complex II polypeptides (C03G5.1 and C34B2.7 are homologous to SDHA, F42A8.2 is homologue to SDHB, F33A8.5 is SDHD and *mev-1* is the structural gene for SDHC (2)). We sequenced several ESTs (thanks to Yuji Kohara) to determine the exon-intron structures of each gene. We used RNAi feeding experiments to study the worm phenotype associated with loss-of-function of worm homologues to the complex II subunits. Our preliminary RNAi experiments, for the anchorage subunits, show a decrease of the progeny numbers that seems to be due to an early arrest of embryo development. The single RNAi experiment for C03G5.1 and C34B2.7 gave no phenotype, whereas the simultaneous RNAi for both genes led to embryonic arrest of development. Thus, the SDHA encoding genes show some functional redundancy. We would like to isolate stable mutants in the genes of interest. Those mutants will be used for complementation experiments with human cDNAs (Wild type and mutants identified in human pathologies). The *mel-9* and *mel-13* mutants mapped very near the physical localization of SDHB and SDHD respectively. We have started to sequence those genes in the respective genetic background to identify any molecular alteration. We also recently received a SDHA mutant harbouring a 1.7 kb deletion in the C03G5.1 gene (thanks to OMRF KO group). We think that the *C. elegans* strains expressing Human complex II subunits we will generate, could be used for future drug screening. We also want to identify genes that are functionally linked to complex II activity. As a first approach we are looking for mutants resistant to complex II inhibitors : theonyltrifluoroacetone (TTFA) and 3-nitropropionic acid (3-NP). We already checked the sensitivity of L1 stage worms for the two drugs, and we found lethal doses of 2.2 mM (for TTFA) and 15 mM (for 3-NP). We also tested TTFA effect on L4 worms and found that they are just as sensitive as the L1. We have recently commenced a genetic screen to select mutants resistant to both inhibitors. (1)Rustin P et al. (2002) Eur J Hum Genet., 10, 10289-10291, (2)Ishii N et al. (1998) Nature 394, 694-697

Comparison of the Complete ATP-Binding Cassette(ABC) transporters of *C. elegans* and *C. Briggsae*: Orthology and Divergence

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ABC transporters constitute one of the biggest gene families in *C. elegans.* They are mostly involved in transmembrane delivery of substrates such as amino acids, peptides and toxins. In order to understand how big gene families are organized and how they evolve, comparative analysis of the ABC transporters has been done on a genome scale between *C.elegans* and *C. briggsae* now that both of genome sequences are available. 59 ABC genes have been identified and classified into 8 different subfamilies in *C.elegans* by a combination of database search and phylogenetic analysis. Two genes, C56E6.5 and C56E6.1, in *C.elegans* , cannot be assigned into established human subfamilies and has been assigned to a new subfamily H. Each subfamily shows similar structure to the equivalent human one. Annotation of *C. briggsae* ABC transporters has been done by database search, *ab initio* gene prediction, and the use of syntenic relationships. It turns out that ABC transporters are well conserved between the two morphologically similar worms. 57 putative orthologous ABC transporters in *C.briggsae*have been identified which can also be grouped into 8 subfamilies, and only 3 of *C. elegans* ABC genes do not have an obvious ortholog in *C. Briggsae*. Most of the *C. briggsae* ABC transporters are of comparable size to their orthologs in *C. elegans*. 6 inversions and 5 deletions/insertions were found among the nearest neighbours of the 57 orthologous pairs. 45 of the 57 *C. Briggsae* orthologous ABC genes were confirmed both by syntenic and phylogenetic analysis. 6 putative *C. briggsae* ABC orthologs assigned by BLAST score don't have any syntenic support, but 4 of them are clustered well with their *C.elegans* orthologues when ABC transporters from both species are used to generate a phylogenetic tree. 14 *C. Briggsae* ABC genes are not supported by the tree data, suggesting possible mis-prediction or sequence error. The transposase or reverse transcriptase insertion were found within two gene clusters in *C. briggsae*, suggesting the role of transposition in the formation of local gene clusters. Two *C. briggsae* ABC genes are assigned only by sequence similarity and have no tree or syntenic support, suggesting transposition or misprediction. No significant ortholog can be found for one of the *C. elegans* ABC transporters, Y57G11C.1. However, as there is no EST match for this gene in the database, it is possible that the gene is a pseudogene, which has therefore undergone a great deal of mutation. Also, local duplication occurs more frequently for ABCs on the sex chromosome than on autosomes. We are investigating the expression pattern of all the ABC transporters in *C.elegans* using GFP as markers, those having the same tissue expression will be used to map transcriptional elements. The orthology information will facilitate the identification of some cis-elements shared between the two species.

An attempt to slow aging in *C. elegans*. 26. A slight positive effect of streptomycin sulphate in concentrations 1.0 and 0.1 mg/ml

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The purpose of this study was to investigate the effect of streptomycin sulphate in water solutions on the nematode life span. In this experiment streptomycin sulphate was used in following dilutions: 1.0 and 0.1 mg/ml. Three adult animals (3 - 5 days old) were kept in microtitre wells containing 0.5 ml of liquid medium (with *E. coli* and without streptomycin sulphate) during 4 hours, then they were discarded and newborn larvae were transferred in next wells (without streptomycin sulphate in medium) every day (one worm in one well) beginning from third day. Then, beginning from 3th day, these worms were transferred every day in next wells containing medium with streptomycin sulphate in any concentration. This investigation was carried out in temperature +21°C and in the darkness.

The obtained results are presented in the following table.

Concentration of streptomycin sulphate (mg/ml)	n	Longevity (days)	
		Mean±S.E.	Maximal
Control	36	10.53±0.90	23
1	36	11.22±1.07	27
0.1	36	12.14±1.17	28

Conclusion: If streptomycin sulphate solution was applied to *C. elegans*, it was able to increase not significantly (P>0.05) their mean longevity in comparison with control to 6.6 and 15.7 percent, respectively.

Acknowledgment: The author wishes to express his thanks to CGC for providing C. elegans (Bristol, N2) and E. coli OP50.

An attempt to slow aging in *C. elegans*. 27. A positive effect of acetylsalicylic acid

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The purpose of this study was to investigate the effect of acetylsalicylic acid in water solutions on the nematode life span. In this experiment acetylsalicylic acid was used in following dilutions: 1000, 100, 10, 1.0 and 0.1 mg/L. Three adult animals (3 - 5 days old) were kept in microtitre wells containing 0.5 ml of liquid medium (with *E. coli* and without acetylsalicylic acid) during 4 hours, then they were discarded and newborn larvae were transferred in next wells (without acetylsalicylic acid in medium) every day (one worm in one well) beginning from third day. Then, beginning from 3rd day, these worms were transferred every day in next wells containing medium with acetylsalicylic acid in any concentration. This investigation was carried out in temperature +21°C and in the darkness. The obtained results are presented in the following table.

Concentration of acetylsalicylic acid (mg/L)	n	Longevity (days)	
		Mean±S.E.	Maximal
Control	12	8.83±1.13	18
1000	12	9.67±1.78	22
100	12	11.50±1.84	26
10	12	16.25±2.28	32
1	12	7.92±0.73	11
0.1	12	14.25±1.72	21

Conclusion: If acetylsalicylic acid solution was applied to *C. elegans* in concentration of 10 mg/L, it was able to increase significantly ($P>0.05$) their mean longevity in comparison with control to 83.96 percent.

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