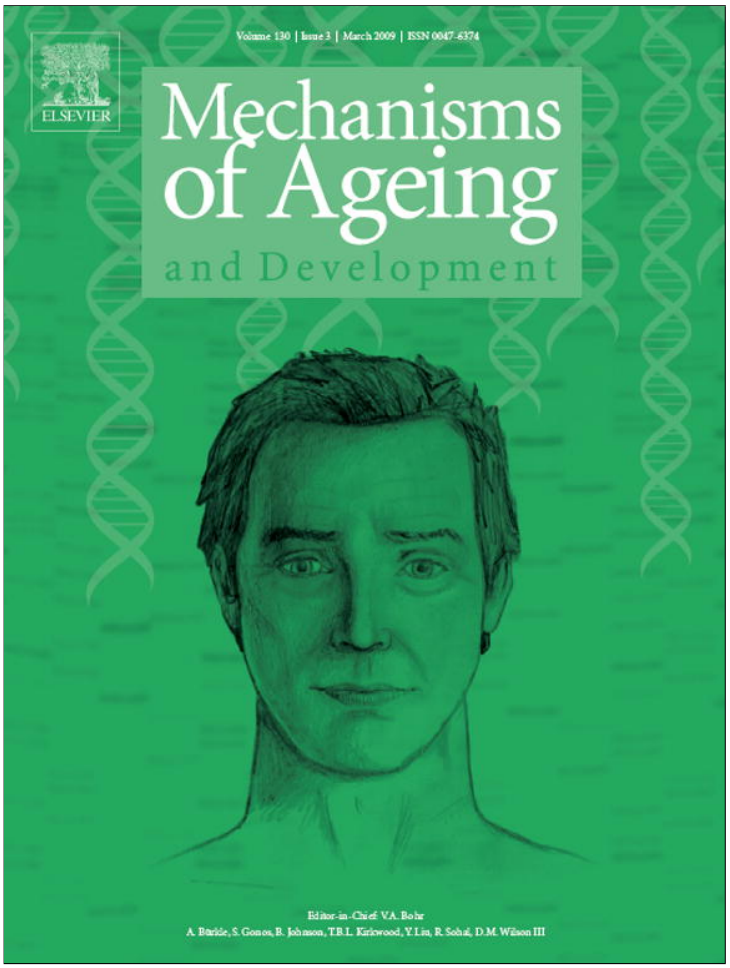

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Transcript profiles of long- and short-lived adults implicate protein synthesis in evolved differences in ageing in the nematode *Strongyloides ratti*☆

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ABSTRACT

The nematode *Strongyloides ratti* shows remarkable phenotypic plasticity in ageing, with parasitic adults living at least 80-times longer than free-living adults. Given that long- and short-lived adults are genetically identical, this plasticity is likely to be due to differences in gene expression. To try and understand how this inter-morph difference in longevity evolved, we compared gene expression in long- and short-lived adults. DNA microarray analysis of long- and short-lived adults identified 32 genes that were up-regulated in long-lived adults, and 96 genes up-regulated in short-lived adults. Strikingly, 38.5% of the genes expressed more in the short-lived morph are predicted to encode ribosomal proteins, compared with only 9% in the long-lived morph. Among the 32 longevity-associated genes there was very little enrichment of genes linked to cellular maintenance. Overall, we have therefore observed a negative correlation between expression of ribosomal protein genes and longevity in *S. ratti*. Interestingly, engineered reduction of expression of ribosomal protein genes increases lifespan in the free-living nematode *Caenorhabditis elegans*. Our study therefore suggests that differences in levels of protein synthesis could contribute to evolved differences in animal longevity.

1. Introduction

The rate of ageing varies greatly between different animal species. For example, different nematodes show maximum lifespans that range from 3 days to at least 15 years (Gems, 2000). This variation reflects evolved differences in genotype, presumably corresponding to differences in both gene expression and the nature of expressed proteins. However, almost nothing is known about the nature of these genes and proteins. In this study, we explore this by means of a special biogerontological model, the parasitic nematode *Strongyloides ratti*.

The life-cycle of this organism includes two adult generations: a parasitic female-only generation and a dioecious free-living male and female generation (Fig. 1). The parasitic generation lives in the small intestine of rat hosts and reproduces by a genetically mitotic parthenogenesis (Viney, 1994, 1999). The free-living adult generation lives in the external environment and reproduces by sexual reproduction (Viney et al., 1993). Senescence occurs in both the parasitic and free-living adults of *S. ratti*, but the former age much more slowly than the latter and, strikingly, show an 80-fold greater longevity, resulting in maximum lifespans of ~1.1 years and 5 days, respectively (Gardner et al., 2004, 2006a).

Parasitic nematodes often evolve lifespans that are much greater than those typical of free-living nematodes (Gems, 2000). This seems likely to reflect the evolutionary consequences of differences in extrinsic mortality hazard associated with the difference in life style (Medawar, 1952). For *S. ratti*, this implies that the extrinsic mortality hazard rate is greater for free-living stages than for parasitic stages within the host, despite the immune response that can act effectively against *S. ratti* in the latter milieu (Wilkes et al., 2007). Interestingly, long- and short-lived *S. ratti* female adults are genetically identical: female first stage larvae can develop into either form of adult depending on environmental cues (Viney, 1994; Harvey et al., 2000). This means that the evolved dramatic difference in lifespan must be determined epigenetically, most likely by differences in gene expression (Gardner et al., 2006a). Thus, by examining the differences in gene expression between long- and short-lived *S. ratti* adults, we can hope to discover genes and biological processes that underlie evolved differences in longevity.

The control of ageing by differential gene expression has been studied in some detail in the short-lived, free-living nematode *C. elegans*. In this organism, reduction of insulin/IGF-1 signalling (IIS) can increase adult *C. elegans* lifespan up to 10-fold (Ayyadevara

☆ The microarray data reported here have been deposited at ArrayExpress (http://www.ebi.ac.uk/microarray-as/aer/entry) with accession number E-TABM-236.

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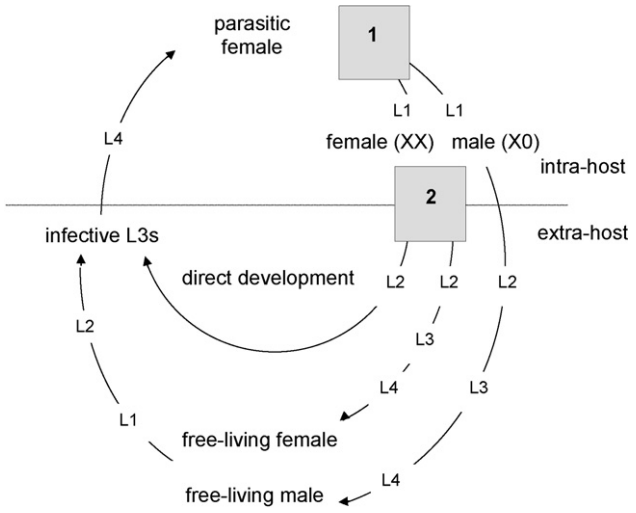

Fig. 1. The life-cycle of *Strongyloides ratti*. The progeny of the parasitic females have two developmental switches (grey boxes): (1) a sex determination event, (2) a female-only developmental switch, after (Harvey et al., 2000). Numbered larval stages are shown as L (Viney and Lok, 2007).

et al., 2008). Microarray studies have shown that, directly or indirectly, IIS regulates expression of several thousand genes in *C. elegans* (McElwee et al., 2004). It is assumed that among these genes are some that directly control the ageing process. One possibility is that IIS might control the difference in longevity between long- and short-lived *S. ratti* adults, perhaps via regulation of similar transcriptional targets to those that so powerfully affect ageing in *C. elegans*.

In this study, we have compared gene expression in long- and short-lived *S. ratti* adults using DNA microarrays. In doing this, we were particularly interested to test for similarities between the sets of genes that are differently expressed in long- vs. short-lived *S. ratti*, and in long-lived mutant vs. short-lived control *C. elegans*. For example, long-lived IIS mutant *C. elegans* show up-regulation of many genes linked to cellular maintenance *e.g.* heat shock proteins (HSP), antioxidant enzymes and drug metabolizing enzymes (McElwee et al., 2003, 2004; Murphy et al., 2003). Somewhat against expectation we detected little up-regulation of cellular maintenance genes in long-lived *S. ratti* adults. However, we did find that many genes predicted to encode ribosomal proteins were more highly expressed in the short-lived adults, implying that the short-lived adults have higher rates of translation and protein synthesis than the long-lived adults. This suggests that higher rates of protein synthesis might lead to shorter lifespan. Consistent with this, several recent studies in *C. elegans* also imply that higher protein synthesis accelerates ageing, by unknown mechanisms, reviewed by (Kaeberlein and Kennedy, 2007). Taken together, this suggests that differences in the rates of protein synthesis may contribute to evolved differences in lifespan.

2. Materials and methods

2.1. Worms

The *S. ratti* isofemale line ED321 Heterogenic (Viney, 1996) was used throughout. Infections were maintained in rats and faecal cultures prepared as described elsewhere (Viney, 1996). Adult parasitic females and adult free-living females were isolated, from which RNA was extracted for use with the microarrays and for reverse transcriptase-PCR (RT-PCR). Adult parasitic females were prepared from *S. ratti* infected rats sacrificed on day 6 post infection (p.i.) and stored as described elsewhere (Thompson et al., 2008). Adult free-living females were prepared by collecting faeces from *S. ratti*-infected rats; these faeces were cultured (Viney, 1996) and held at 19 °C for 3 days. At this time, young free-living males and females, together with various larval stages are present in the liquid surrounding the faecal mass. These worms were removed with a pipette to fresh distilled water. Individual adult free-living females were selected and isolated using a micropipette. Free-living worms collected in this way were stored as for the parasitic females (above).

2.2. Microarray analysis

The *S. ratti* expressed sequence tag (EST) analysis and the consequent production of the *S. ratti* microarrays has been described elsewhere (Thompson et al., 2005, 2006, 2008). Briefly, approximately 20,000 sequence reads resulted in 14,761 ESTs being obtained from five cDNA libraries from the free-living (L1, L2, and mixed iL3/free-living adult) and parasitic (parasitic females 6 and 15 days p.i.) phases of the *S. ratti* life-cycle. These ESTs were grouped into 5237 contigs each of which contained apparently identical or over-lapping ESTs. These contigs were further grouped into 4152 clusters, which are likely to mostly represent single genes. In the main text we refer to *S. ratti* clusters as 'genes'. In previous work we annotated these contigs and clusters by the comparison of sequences against a range of databases (Thompson et al., 2005). Here we have updated this analysis by seeking significant alignments (BLAST with an *E*-value of $<1 \times 10^{-05}$) against the GenBank non-redundant peptide database in September 2007. These alignments are reported here and may, therefore, differ from those previously reported (Thompson et al., 2005). We characterised contigs in this way: to annotate multiple-contig clusters the most significant BLAST alignment of a single representative contig of each cluster was used. In all 1459 (35%) of the 4152 clusters have no significant BLAST alignments. We also annotated *S. ratti* contigs and clusters with Prosite and GO terms (see Supplementary Table). To do this *S. ratti* cluster sequences were matched to a local copy of the Prosite database (ftp://ftp.expasy.org/databases/prosite/prosite.dat) using the ps_scan.pl tool available from ftp://www.expasy.org/databases/prosite/tools/. High frequency patterns were ignored. Protein sequences representative of GO terms were downloaded in FASTA format from http://archive.geneontology.org. The FASTA file was formatted and searched locally using the formatdb and blastall tools available from http://www.ncbi.nlm.nih.gov. BLASTX was used to compare *S. ratti* nucleotide sequences with the GO protein sequence database using an *E*-value cut-off of 10^{-5}. Prosite and GO matches were parsed using custom PERL scripts.

These ESTs were used to construct two microarrays: a free-living microarray consisting of all ESTs sequenced from the free-living stages(Thompson et al., 2006) and a parasitic microarray consisting of all ESTs sequenced from the parasitic female stages (Thompson et al., 2008). The free-living microarray consists of 7182 arrayed *S. ratti* ESTs, representing 2742 contigs and 2590 clusters; the parasitic microarray consists of 7385 arrayed ETSs, representing 2963 contigs and 2125 clusters (Thompson et al., 2006, 2008). There is considerable overlap in the contig representation of the two chips; more than 60% of the sequences present on each chip belong to contigs that are also represented on the other chip. The preparation of probes and hybridisation to the arrays and data acquisition and analyses are all as previously described (Thompson et al., 2006, 2008). These ESTs have also been used in other *S. ratti* microarrays that have compared gene expression in different life-cycle stages (Evans et al., 2008).

2.3. Experimental design

We compared gene expression between the short-lived free-living females and the long-lived parasitic females separately on both the free-living microarray and on the parasitic female microarray. For each of these experiments on the free-living microarray and on the parasitic microarray at least three biological replicates of each sample were used, (*i.e.* there were three separate preparations of free-living and parasitic worms and subsequent RNA preparations) and at least three independent technical replicates (*i.e.* separate cDNA synthesis, amplification and hybridization) of each biological replicate. For each hybridisation a dye-swap was used. All of these procedures were as previously performed (Thompson et al., 2006, 2008).

2.4. Data analysis

Data obtained from the free-living microarray and from the parasitic microarray were treated separately. For each the first round data analysis including background adjustment and quality control were performed as previously described (Thompson et al., 2006, 2008). For each microarray we sought *S. ratti* contigs and clusters whose ratio of expression was significantly different between free-living and parasitic females, and where the fold difference was greater than or equal to 1.5. These analyses were performed analogously to previous analyses (Thompson et al., 2006, 2008). To compare the number of ESTs belonging to clusters with significant alignments to (i) ribosomal proteins, (ii) elongation factors or (iii) heat shock proteins, we selected the clusters with these annotations, determined the number of component ESTs obtained from the free-living and parasitic source libraries (Thompson et al., 2005) and compared these to the null hypothesis of equal representation by χ^2 analysis.

2.5. RT-PCR

We used semi-quantitative reverse transcriptase (RT)-PCR to measure the relative mRNA concentrations of a sample of contigs in parasitic and free-living females. We undertook this analysis on a range of *S. ratti* contigs (many with BLAST

alignment to ribosomal proteins), including those which the microarray data identified as being significantly differently expressed and those which the microarray data did not identify as being significantly differently expressed. In presenting these data (Table 3) we have given the cluster designation and BLAST annotation of the respective contig. These RT-PCR analyses were performed as previously described (Thompson et al., 2006, 2008) using *S. ratti act-3* to normalise for the quantity of cDNA used in each experiment. cDNA was prepared from each of one preparation of free-living or parasitic female worms; three replicate RT-PCR amplifications were performed on each cDNA sample, except for SR00943.cl amplified from parasitic female cDNA, where only two replicate RT-PCRs were performed.

3. Results

3.1. Gene expression in long-lived vs. short-lived S. ratti adults

We used DNA microarray analysis to compare gene expression in long-lived, parasitic and short-lived, free-living adults of *S. ratti*. These *S. ratti* EST-based microarrays represent 4152 clusters of ESTs, where each cluster is likely to represent a single gene (Thompson et al., 2005, 2006, 2008). If the *S. ratti* genome encodes the same number of genes as the *c.*19,000 genes of *C. elegans* (*C. elegans* Sequencing Consortium, 1998), then these 4152 genes are likely to represent *c.*21% of the genes of *S. ratti.* We used these arrays to detect *S. ratti* genes whose expression was significantly different and where the fold difference was greater than or equal to 1.5 between the two *S. ratti* morphs.

Based on current theories of ageing and the results of earlier microarray studies using model organisms, our expectation was that the longevity of *S. ratti* parasitic adults might be attributable largely to up-regulation of genes encoding components of cellular maintenance processes (*e.g.* antioxidant defence, heat shock proteins, autophagy *etc.*) However, most of the genes identified as significantly differentially expressed were up-regulated in the short-lived, free-living adults. Overall, 96 *S. ratti* genes (2.3% of all the clusters on the array) were highly expressed in the short-lived adults compared with the long-lived adults (Table 1). By contrast, only 32 genes (0.8% of all the clusters on the arrays) were more highly expressed in the long-lived adults compared with the short-lived adults.

These results are from the combined analysis of two microarray chips, one for *S. ratti* free-living stages and one for parasitic stages (Thompson et al., 2006, 2008). We detected similar differences in gene expression on both microarrays (Table 1) which implies that there is no bias in the results derived from either array. For the genes that were expressed at significantly greater levels in the short-lived free-living females, the maximum fold-difference in mRNA abundance was 6.3 (median 2.0, mean 2.4, standard deviation (SD) 0.99) and 9.9 (median 3.16, mean 3.85, SD 2.29) as measured using the free-living and parasitic microarray, respectively. For genes that were expressed at significantly greater levels in the long-lived parasitic females, the maximum fold-difference in mRNA abundance was 2.15 (median 1.67, mean 1.69, SD 0.18) and 20.4 (median 1.59, mean 3.4, SD 5.6) as measured using the free-living and parasitic microarray, respectively (see Supplementary Table).

The majority (75%) of these differentially expressed genes (Table 1) showed significant similarity to genes previously identified in other organisms. This is of some note because *c.* 35% of all *S. ratti* genes are, apparently unique, *i.e.* they have no significant sequence alignments (Thompson et al., 2005). This therefore suggests that the apparently very different biology of these two morphs (*e.g.* long- vs. short-lived; animal parasitic vs. free-living) may largely determined by genes that are conserved with other species and therefore possibly relatively evolutionarily ancient.

3.2. Greater expression of genes encoding ribosomal proteins in short-lived adults

38.5% of genes whose expression was greater in short-lived adults are predicted to encode ribosomal proteins, whereas only 2% of all *S. ratti* genes represented on the microarray are predicted to encode ribosomal proteins. This was by far the largest class of genes with this pattern of expression. In contrast, only 9% of genes with greater expression in the long-lived adults are predicted to encode ribosomal protein genes (Table 1, Supplementary Table). The 95% confidence intervals for these proportions of ribosomal protein genes do not overlap. These genes of the short-lived adults encoded putative homologues of various components of both the large and small ribosomal sub-units (Table 2). These results imply that there is greater transcription of many ribosomal protein genes in the short-lived adults compared with the long-lived adults.

We used semi-quantitative RT-PCR to confirm the microarray data. This showed for a range of *S. ratti* genes predicted to encode ribosomal proteins, that expression was greater in short-lived adults, compared with long-lived adults (Table 3). This was observed both for genes identified from the microarray data as being significantly differentially expressed and for some that were not so identified. This therefore suggests that our microarray data are conservative in their measurement of differential gene expression, as has been observed previously (Thompson et al., 2006, 2008).

Existing data on abundance of expressed sequence tags from the *S. ratti* short-lived stages and from the long-lived parasitic

Table 1
The total number of unique genes that are significantly differently expressed at > 1.5-fold in free-living females and in parasitic females, on the parasitic microarray and the free-living microarray and these combined, and the number that have significant BLAST alignment to ribosomal proteins with, in parentheses, this expressed as a percentage with 95% confidence intervals (Rohlf and Sokal, 1969) shown below.

	Free-living females-up		Parasitic females-up	
	Total	Ribosomal	Total	Ribosomal
Parasitic microarray	64	27 (42%)	11	0 (0%)
Free-living microarray	51	21 (41%)	22	3 (14%)
Total	115	48 (42%) (33–51%)	33	3 (9%) (2–26%)
Total unique	96	37 (38.5%) (29–48%)	32	3 (9%) (2–26%)

Table 2
The large and small ribosomal subunit proteins which are significant BLAST alignments to the *S. ratti* genes that are significantly differently expressed (≥1.5-fold) in free-living females, compared with parasitic females, identified from the parasitic (P) or free-living (F) microarray indicated.

Large subunit	Small subunit
6 (P)	1 (P)
7 (P)	5 (F)
9 (F)	8 (F)
10 (F)	11 (P)
11.2 (P*)	12 (F, P)
14 (F, P)	14 (F, P)
15 (F, P)	18 (F)
16 (P)	19S (F, P)
18e (P)	20 (F)
22 (P)	21 (P)
23 (F)	23 (P)
23a (F)	24 (F)
29 (F, P)	25 (P)
30 (F, P)	26 (F, P)
31 (P)	29 (F)
35 (P)	
35a (F)	
36 (F)	
37a (P)	
39 (P)	

* Two genes had significant BLAST alignments to these proteins.

Table 3
A comparison of RT-PCR and microarray measures (from the free-living (F) and parasitic (P) microarrays) of gene expression, calculated as free-living-female/parasitic female. The BLAST annotations of the respective genes are shown (see Supplementary Table).

Gene	RT-PCR (± 1SE)	Microarray	BLAST annotation
SR01066.cl	2.06 (0.14)	5.06 (P)	RPL 16
SR00953.cl	6.03 (0.37)	4.53 (P)	RPL 37a
SR00915.cl	5.14 (0.27)	9.58 (P)/2.91 (F)	RPL 29
SR00943.cl	5.98 (0.73)	9.38 (P)/3.89 (F)	RPL 14
SR00704.cl	5.25 (0.25)	5.74 (P)	Ce hyp
SR00982.cl	6.56 (0.52)	4.76 (P)/2.11 (F)	RPS 12
SR00978.cl*	3.81 (0.70)	–	RPS 13
SR00783.cl	9.01 (2.41)	3.81 (P)	RPS 21
SR00211.cl	6.21 (1.56)	7.70 (P)	RPL 31
SR01040.c	3.92 (0.10)	3.97 (P)	RPS 23
SR01520.cl	1.25 (0.56)	2.06 (F)	Ce *nft-1*
SR00940.cl*	1.49 (0.067)	1.07 (F)	Sp hyp
SR00007.cl*,†	0.17 (0.019)	0.88 (P)	No BLAST

* Genes where the difference in the measured microarray expression was not statistically significantly different.
† Gene with greater expression in parasitic females, hence expression ratios of less than 1. RPL – large ribosomal subunit, RPS – small ribosomal subunit, Ce – *Caenorhabditis elegans*, SP – *Schizosaccharomyces pombe*, hyp – hypothetical protein, – no data, *nft-1* – NitFhit family-1.

female stages (Thompson et al., 2005) provides further information about inter-morph differences in gene expression in this organism. We examined these data for further evidence of increased expression of genes associated with protein synthesis in the short-lived, free-living adults. Significantly more ESTs corresponding to genes predicted to encode ribosomal proteins (1491 and 281 ESTs of 74 genes from short- and long-lived adult female stages, respectively, χ^2 (1, $N = 14{,}701$) = 927, $p < 0.001$) were present among ESTs obtained from short-lived adult stages, compared with those obtained from long-lived adult stages. We also found that there were significantly more ESTs corresponding to genes predicted to encode elongation factors (105 and 44 ESTs of 9 genes from short- and long-lived adult female stages, respectively, χ^2 (1, $N = 14{,}701$) = 25, $p < 0.001$) present among ESTs obtained from short-lived adult stages, compared with those obtained from long-lived adult stages. However, the microarray data did not detect this pattern; rather, it showed that one gene predicted to encode an elongation factor was expressed at significantly greater levels in long-lived adults, compared with short-lived adults.

3.3. No clear increase in cellular maintenance or transcriptional signature of IIS regulation in long-lived adults

Potentially, the greater longevity of parasitic *S. ratti* adults could result from increased expression of genes specifying cellular maintenance processes, perhaps as the result of down-regulation of IIS. In *C. elegans*, reduced IIS leads to increased expression of genes encoding various classes of protein, including small HSP, antioxidant enzymes (superoxide dismutase, catalase) and drug metabolizing enzymes (DMEs, *e.g.* cytochrome P450 oxidases and glutathione S-transferases). To investigate this we asked whether genes and gene classes previously identified to have altered expression in two *C. elegans* forms with increased lifespans (IIS mutants adults and dauer larvae) occurred among the *S. ratti* putative lifespan-associated genes. We did this using the annotation terms and gene ontology (GO) classes defined by (McElwee et al., 2004), compared to the BLAST, Prosite and GO annotations of the *S. ratti* genes (see Supplementary Table). In this way we identified 194 *S. ratti* genes present on the microarrays that matched at least one of these terms (McElwee et al., 2004) including several classes of enzymes such as short chain dehydrogenase/reductase, carboxylesterase, UDP- glucosyltransferase, thioesterase, glutathione s-transferase and superoxide dismutase and catalase. This suggests that the *S. ratti* microarrays would be able to detect any significant changes in expression of these cellular maintenance-associated genes. However, among the total 128 differentially expressed genes (96 short-lived and 32 long-lived, Table 1) only eight matched terms for cellular maintenance (McElwee et al., 2004). Two of these (GO:0006508, GO:0006810) were genes more highly expressed in the short-lived, free-living females. Five of these (GO:0016491, GO:0006508, thioesterase, two HSP) were genes expressed comparatively more in the long-lived parasitic females. We considered the HSPs of *S. ratti* further, by comparing the distribution of *S. ratti* ESTs that correspond to genes predicted to encode HSP. This showed that there were significantly more ESTs predicted to encode HSP from the parasitic stages, compared with free-living stages (175 and 51 ESTs of 11 genes from parasitic and free-living stages, respectively; χ^2 (1, $N = 14{,}701$) = 70, $p < 0.001$). Thus, our microarray analysis did not detect a clear transcriptional signature of reduced IIS or increased somatic maintenance in the long-lived adult. However, EST abundance analysis suggested increased expression of HSP in long-lived adults. HSP contribute to somatic maintenance (protein folding homeostasis), and in *C. elegans* have been shown to be up-regulated in long-lived IIS mutants. Moreover, over-expression of HSP can increase lifespan (Walker and Lithgow, 2003). Thus, their up-regulation in long-lived adults is at least consistent with a role in longevity assurance, and IIS regulation.

4. Discussion

The results of this microarray study suggest that differences in levels of protein synthesis may contribute to the striking, evolved difference in longevity between long-lived parasitic and short-lived free-living *S. ratti* adults. This is supported by analysis of EST representation between short- and long-lived adult stages; this analysis also suggests that the expression of HSP coding genes may also contribute to long lifespan. It is also consistent with recent work on a related nematode, *Parastrongyloides trichosuri*, a parasite of Australian brush-tail possums with a life-cycle similar to that of *S. ratti*. This nematode also shows a large difference in lifespan between parasitic and free-living adults (Grant et al., 2006). Microarray comparisons of mRNAs between long- and short-lived adults also shows reduced expression of many ribosomal protein genes in the long-lived adults (W.N. Grant, personal communication). Thus, our findings at least appear generalizable within the Strongyloididae nematodes.

Consistent with this, recent studies in *C. elegans* imply that higher rates of protein synthesis accelerate ageing (Henderson et al., 2006; Hansen et al., 2007; Pan et al., 2007; Syntichaki et al., 2007) reviewed by Kaeberlein and Kennedy (2007). In these studies a range of manipulations were used to reduce components of protein synthesis processes, including targeted reduction of expression of ribosomal protein and elongation factor genes. Comparison of these findings together with our observations, suggest that protein synthesis may also be related to evolved differences in lifespan. The mechanism by which lowered protein synthesis increases lifespan remains unclear. One suggestion is that the proteasomal protein degradation capacity of cells is suboptimal such that damaged proteins and misfolded nascent proteins compete for access to the proteasome. Thus, lowering the rate of protein synthesis increases the capacity of proteasomes to degrade proteins whose accumulation otherwise contributes to ageing (Hipkiss, 2007).

Analysis of EST representation, but not microarray data, implies that there is greater expression of HSP coding genes in long-lived adults. This is interesting because *C. elegans* lifespan can be

increased by over-expression of the transcription factor HSF-1 (Hsu et al., 2003), which controls HSP gene expression, and by small HSPs (Walker and Lithgow, 2003). This is consistent with the view that progressive loss of protein folding homeostasis (proteostasis) contributes to ageing. Thus, contribution of increased HSP expression to the longevity of long-lived *S. ratti* is plausible.

At the outset of our investigation, we had postulated that ageing in *S. ratti* might be regulated by IIS, perhaps including the *S. ratti* orthologue of the transcription factor DAF-16/FOXO, which is required for the effects of IIS on ageing in *C. elegans* (Kenyon et al., 1993. The putative orthologue of *daf-16* has been identified in the human parasitic species *S. stercoralis* (Massey et al., 2003). Studies of gene expression in *C. elegans* IIS mutants have shown increased expression of various classes of genes linked to cellular maintenance (McElwee et al., 2003, 2004; Murphy et al., 2003). In this study, we did not see widespread up-regulation of genes of this sort in long-lived *S. ratti* adults. This does not obviously seem to be due to the lack of sensitivity of our methodologies. Our results therefore suggest that in *S. ratti* IIS is not important in regulating lifespan.

However, it is worth noting that a recent study comparing transcript profiles from long-lived mutant *C. elegans*, fruit flies (*Drosophila melanogaster*) and mouse, all with reduced IIS, detected significant down-regulation of genes linked to protein synthesis in all three cases (McElwee et al., 2007). Thus, the apparent reduction of protein synthesis in parasitic *S. ratti* adults is also consistent with reduced IIS. However, we have no direct evidence that IIS is reduced in long-lived *S. ratti* adults, and other pathways could well be critical. For example, in model organisms from budding yeast (*Saccharomyces cerevisiae*), to *C. elegans*, to *D. melanogaster*, protein synthesis and lifespan are both regulated by the Tor (Target of rapamycin) pathway (Kaeberlein and Kennedy, 2007). Our findings suggest the possibility that Tor signalling may be reduced in parasitic *S. ratti* adults.

Our results have found a correlation between the expression of ribosomal protein coding genes and short lifespan. However, there are other differences between the adult morphs of *S. ratti*. Thus, the long-lived morph is also parasitic, reproduces by parthenogenesis and has a lifetime fecundity of *c.*16,000 (Wilkes et al., 2007). The short-lived morph is also free-living, reproduces sexually and has a lifetime fecundity of *c.*40. The differences in gene expression that we have observed between these morphs could be due to some of these other differences between these morphs. However, it is difficult to understand how the comparative 400-fold lower fecundity of the free-living females is related to lower expression of ribosomal protein coding genes.

A useful feature of *S. ratti* as a model organism is that it is, as it were, two different organisms with two different niches (parasitic and free-living) and two lifespans, but specified by a single genome. Thus, the determinants of differential longevity in this species can, potentially, provide insight into the biological mechanisms that specify differences in longevity between animal species (Gardner et al., 2006a,b). A number of comparative studies of animal ageing have sought to understand such variation, largely without success. Subjects for investigation include the differences in longevity between castes of eusocial insects such as the ant, *Lasius niger* (Parker et al., 2004), the longevity of the longest lived rodent, the naked mole-rat *Heterocephalus glaber* (Buffenstein, 2008), and the striking longevity of humans relative to other higher primates, particularly the very closely related common chimpanzee, *Pan troglodytes* (de Magalhães and Church, 2007). Our findings suggest the possibility that lowered rates of protein synthesis and increased levels of HSPs might contribute to the relative longevity in other species, such as ant queens, naked mole-rats and/or humans.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.mad.2008.11.001.

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