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Mini Review

Alternate metabolism during the dauer stage of the nematode *Caenorhabditis elegans*

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Abstract

When environmental conditions are unsuitable to support nematode reproduction, *Caenorhabditis elegans* arrests development before the onset of sexual maturity and specialised 'dauer' larvae, adapted for dispersal, and extended diapause are formed. Dauer larvae do not feed and their metabolism is dependent on internal food reserves. Adult worms which express defects in the insulin/insulin-like growth factor receptor DAF-2 also display enhanced longevity. Whole genome mRNA expression profiling has demonstrated that *C. elegans* dauer larvae and *daf-2* adults have similar transcription profiles for a cohort of longevity genes. Important components of this enhanced longevity system are the α-crystallin family of small heat shock proteins, anti-ROS defence systems, increased activity of cellular detoxification processes and possibly also increased chromatin stability and decreased protein turnover. Anaerobic fermentation pathways are upregulated in dauer larvae, while long-lived *daf-2* adults appear to have normal oxidative metabolism. Anabolic pathways are down regulated in dauer larvae (and possibly in *daf-2* adults as well), and energy consumption appears to be diverted to enhanced cellular maintenance and detoxification processes in both systems.

Keywords: Dauer; Gene expression profiling; *daf-2*; Metabolism; Malate dismutation; ROS; α-crystallin; Detoxification; Longevity

1. Introduction

When growth conditions are favourable, the nematode *Caenorhabditis elegans* develops rapidly from fertilised eggs through four larval stages (L1–L4) to become an adult hermaphrodite within 3 days. Each self-fertile hermaphrodite is capable of producing up to 280 progeny, so high population densities are rapidly attained. When environmental conditions are unsuitable to support nematode reproduction, *C. elegans* development arrests before the onset of sexual maturity and specialised 'dauer' larvae, adapted for dispersal and extended diapause are formed. These dauer larvae are highly modified L3 larvae. They do not feed; their intestine is sealed off at the mouth and anus; their cuticle is strengthened and resistant to treatment with harsh chemicals; their metabolic activity is reduced and their metabolism is dependent on internal energy stores; they are resistant to environmental stresses such as heat, cold, oxidative stress and desiccation, however their chemosensory system is functional and they are capable of rapid movement (Cassada and Russell, 1975; Riddle and Albert, 1997). These adaptations enable dauer larvae to survive for over 3 months, whereas the normal adult lifespan is ca. 14 days. When dauer larvae locate a suitable food source they resume development, moult to the L4 larval stage and proceed to adulthood.

A *Caenorhabditis*-specific pheromone, constitutively produced by all developmental stages of the nematode, serves as an indicator of population density. The chemical structure of this pheromone has been elucidated recently (Jeong et al., 2005). A build up of this dauer inducing pheromone is the normal physiological trigger for dauer formation. Temperature and bacterial food-signals modulate the response to pheromone at intermediate pheromone concentrations, with higher environmental temperatures favouring dauer formation. Under dauer-inducing conditions a morphologically distinct 'predauer' L2d larva is formed (Riddle and Albert, 1997). L2d larvae prolong the second larval stage and prepare to enter the dauer diapause by accumulating food reserves in their intestinal and hypodermal cells (Fig. 1). Environmental cues are integrated via *da*uer *f*ormation (*daf*) genes (Albert and Riddle, 1988). These genes define the signalling pathways which integrate information from environmental cues to

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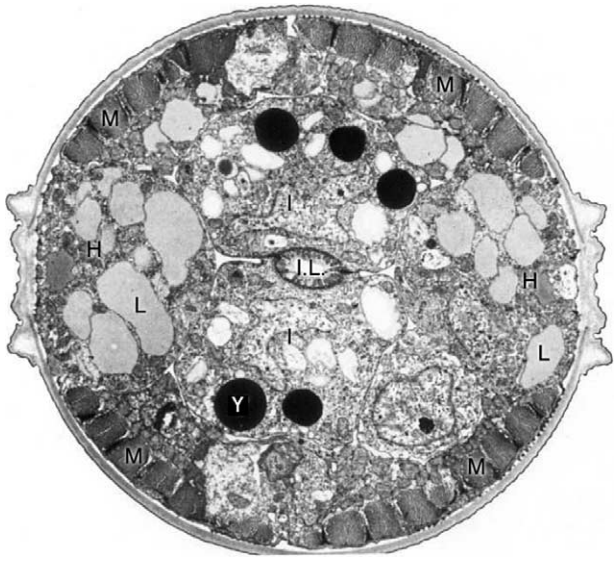

Fig. 1. Transverse section through the intestinal region of a dauer larva of *Caenorhabditis elegans*. I. intestinal cell; I.L. intestine lumen; H. lateral hypodermis; M. muscle; L. lipid; Y putative yolk granules (David Hall, personal communication; see also Hall et al., 1999). Image reprinted from Development 126 (2), Albert, P.S., and Riddle, D.L., Mutants of *Caenorhabditis elegans* that form dauer like larvae, pages 270–293, copyright (1988), with permission from Elsevier.

control the dauer versus non-dauer developmental decision. Identified pathways regulating this decision include insulin/IGF-1, TGF-β and cyclic GMP signalling. These signal transduction pathways converge on DAF-9, a cytochrome P450 steroid hydroxylase which is postulated to produce a cholestrol-derived hormone (Gerish and Antebi, 2004; Matyash et al., 2004). The target of this putative hormone is the nuclear hormone receptor DAF-12. Hormone activated DAF-12 promotes reproductive development, while in the absence of this hormone DAF-12 activates the dauer formation programme. Expressing *daf-9* constitutively in the hypodermis rescues the dauer arrest phenotypes of mutants defective in insulin/IGF-1 or TGF-β signalling, providing evidence that *daf-9* integrates the outputs from these signalling pathways. However, some residual defects are observed in the rescued worms, suggesting that the dauer signalling pathways may also have some *daf-9* independent outputs (Gerish and Antebi, 2004).

In 1993, Kenyon et al. (Kenyon et al., 1993) discovered that the life maintenance programme of dauer larvae can be ectopically expressed in adult worms. Mutations in the *daf-2* gene induce inappropriate dauer formation in the presence of abundant food (Albert and Riddle, 1988). Growing temperature sensitive *daf-2* mutants at the permissive temperature (15 °C) until they had reached the L4 stage and then upshifting them to the restrictive temperature (25 °C) gave rise to adult worms whose lifespan was over twice as long as wild type worms. This lifespan extension was suppressed by mutations in the *daf-16* gene. The *daf-2* gene encodes an Ins/IGF-1-like receptor and *daf-16* encodes a forkhead transcription factor. In the absence of insulin signalling from the *daf-2* pathway, DAF-16 resides in the nucleus and modulates transcription, but when phosphorylated as a result of Ins/IGF-1 signalling, DAF-16 relocates to the cytoplasm and is inactive (Henderson and Johnson. 2001).

Dauer larvae are a key component of the survival and dispersal strategy of *C. elegans* and such larvae also occur in many other free living and parasitic nematodes. Dauer formation and exit/recovery from the dauer state are tightly regulated in order to balance the utilisation of local food resources with the needs of an expanding population to disperse and forage for food. Thus traits which refine the sensory transduction pathways controlling dauer formation, or which enhance the longevity phenotypes of these pre-reproductive developmental stages, are likely to be subject to strong selection pressure. Here, we review the current state of knowledge of intermediary metabolism of the *C. elegans* dauer larva, with particular emphasis on those aspects of metabolism and cell biology which may contribute to dauer longevity.

2. Intermediary metabolism

The metabolism of dauer larvae is adapted to utilise internal energy reserves, predominantly lipid in the form of triglycerides, but also glycogen. Nevertheless, oxidative metabolism is lower in dauers than in mixed stages or adults (O'Riordan and Burnell, 1989; Vanfleteren and de Vreese, 1996). An overview of the main pathways for energy metabolism which have the potential to be active in dauer larvae is presented in Fig. 2. The glycerol moiety of triglycerides along with glucose-1-phosphate molecules arising from glycogen breakdown are metabolised through glycolysis, while fatty acids are metabolised through the β-oxidation pathway. In *C. elegans* β-oxidation occurs in the mitochondria and also in peroxisomes (Gurvitz, 2000). *C. elegans* dauer larvae possess a functional glyoxylate cycle. The glyoxylate cycle provides a means for the conversion of 2-carbon acetylCoA units derived from the β-oxidation of fatty acids into the 4-carbon molecules succinate and malate, which can then be used as precursors in the biosynthesis of carbohydrates and other cellular components. In plants and yeast the enzymes of the glyoxylate cycle are compartmentalised into organelles called glyoxysomes, and there is also evidence from subcellular fractionation experiments using isopycnic sucrose gradients that *C. elegans* also contains glyoxysome like microbodies (Patel and McFadden, 1977).

Genome wide analyses of mRNA transcript abundance in *C. elegans* dauer larvae have been carried out using two approaches. Serial analysis of gene expression (SAGE) of dauer larvae and non-dauer mixed stage populations was carried out by Jones et al. (2001); Holt and Riddle (2003). Wang and Kim (2003) used DNA microarray analysis to profile gene expression differences during the dauer state and the transition to dauer recovery and they also investigated the gene expression profiles obtained after feeding starved L1 larvae. Although mRNA expression levels cannot be consistently relied on as a predictor of protein abundance, the data obtained by gene expression profiling compared well with earlier biochemical assays. These global profiles have provided

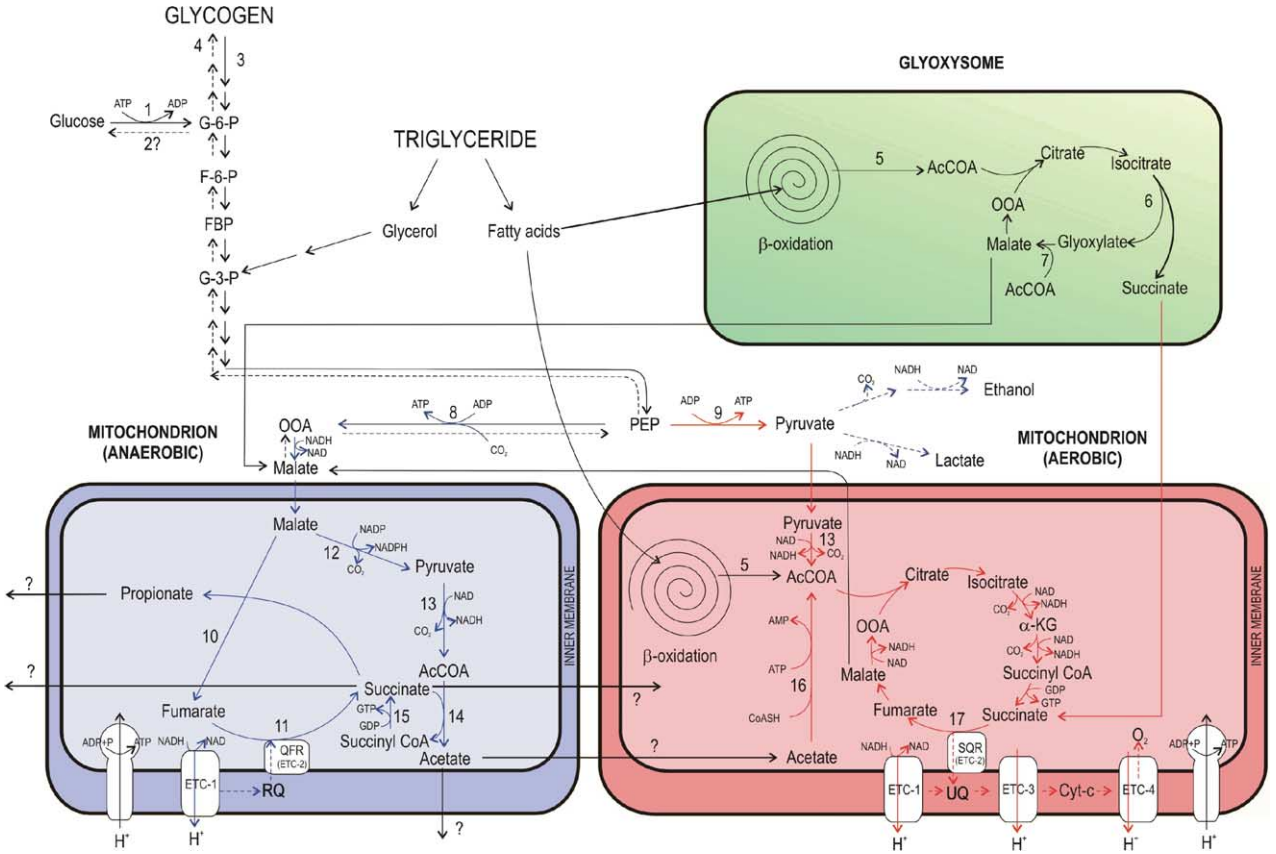

Fig. 2. Overview of the pathways involved in carbohydrate and lipid metabolism in the dauer larva of *C. elegans*. Aerobic and anaerobically functioning mitochondria are illustrated separately for clarity, but it is likely that an individual mitochondrion may function aerobically or anaerobically depending on the availability of oxygen. Some key enzymes are indicated: 1, hexokinase; 2, a distinct glucose-6-phosphatase has not been detected in *C. elegans* (Wang and Kim, 2003); 3, glycogen phosphorylase; 4, glycogen synthase; 5, 3-ketoacyl CoA thiolase; 6, isocitrate lyase; 7, malate syntase; 8, phosphenol pyruvate carboxykinase; 9, pyruvate kinase; 10 fumarase (fumarate hydratase); 11, fumarate reductase; 12, malic enzyme; 13, pyruvate dehydrogenase; 14, putative acetate-succinate CoA transferase [C05C10.3]—SAGE tags for this transcript are represented in the dauer library (http://elegans.bcgsc.ca/home/sage.html); 15, succinyl CoA synthase; 16, putative acetylCoA synthase [C36A4.9]—the activity of this transcript is elevated in dauer larvae relative to the end of the dauer recovery time-course (http://cmgm.stanford.edu/%7Ekimlab/dauer/SearchDauer.htm); 17, succinate dehydrogenase.

evidence for upregulation in dauer larvae of several anaerobic pathways including the 'malate dismutation' and fermentation pathways. The malate dismutation process enables mitochondria to function anaerobically and requires the use of a specialised electron transport chain. Parasitic helminths that encounter very low oxygen pressures in their habitat typically rely on this pathway for their energy metabolism (Tielens et al., 2002). Holt and Riddle (2003) have postulated that a gradient of oxygen availability from the body surface to interior of the intestine exists in dauer larvae. This gradient could arise from the sealed intestine, dense body and relatively impermeable cuticle of dauer larvae. An hypoxia gradient from the interior to the exterior of dauer larvae may result in anaerobic metabolism occurring in the mitochondria of the intestine, with the transport of fermentation products and the products of the glyoxylate cycle to the peripheral tissues (muscles and hypodermis) for aerobic metabolism and gluconeogenesis.

2.1. Fermentation pathways in C. elegans dauer larvae

The phosphoenolpyruvate (PEP) resulting from glycolyis provides an important branch point between aerobic and anaerobic mitochondrial function. PEP may be converted to pyruvate by pyruvate kinase (PK) or to oxaloacetate (OOA) using phosphenolpyruvate carboxykinase (PEPCK). Malate is formed from OOA and enters anaerobically functioning mitochondria for further degradation, while pyruvate may enter aerobic mitochondria for oxidative decarboxylation to acetylCoA followed by entry into the tricarboxylic acid cycle. Alternatively pyruvate may be reduced anaerobically in the cytosol to lactate or ethanol. SAGE analysis revealed that dauer larvae had over twice as many transcripts for PEPCK than for PK (112 vs 46), suggesting that the formation of OOA may be favoured. In anaerobically functioning mitochondria malate is degraded by one of two routes: it may be oxidised via pyruvate and acetylCoA to acetate or reduced via fumarate to succinate at complex II of the electron transport chain. Two molecules of NAD(P)H are generated in the oxidation of malate to acetate and one molecule of NADH is reoxidised to NAD in the reduction of fumarate to succinate. Thus these mitochondrial reactions achieve redox balance in the absence of O_2 if acetate and succinate are produced in a ratio of 1:2 (see Fig. 2). The reduction of malate to succinate is achieved by the reverse action of fumarase and complex II of the electron transport chain. Complex II can act as succinate dehydrogenase or as fumarate reductase depending on the properties of some of its polypeptide subunits and the presence or absence of the electron transporter rhodoquinone (RQ). RQ has a lower reduction potential than ubiquinone (Tielens et al., 2002) permitting electron flow from succinate to fumarate. Based on these redox potentials, the presence of rhodoquinone is sufficient to turn complex II succinate dehydrogenase (succinate:ubiquinone oxidoreductase, SQR) into a fumarate reductase (quinol:fumarate oxidoreductase, QFR), but specific adaptations of complex II subunits may also facilitate this reversal. *C. elegans* expresses two distinct complex II flavin-containing (Fp) subunits and the expression of one of these remains unchanged in dauers while the other undergoes a 10-fold reduction (Rea and Johnson, 2003). The dauer larva of *C. elegans* expresses all essential components needed for functional malate dismutation and excretion of propionate, including the electron transporter RQ (Holt and Riddle, 2003; Takamiya et al., 1999). A cytosolic fumarate reductase is also enriched in dauers (Holt and Riddle, 2003).

Dauer larvae of *C. elegans* are more resistant to hypoxic stress than adults or other larval stages. However, in our experience dauer larvae are unable to survive anoxia much longer than 24 h at room temperature. Thus the dauer larva is specialised to survive short periods of severe hypoxia or longer periods of mild hypoxia, and it has an absolute requirement for oxidative metabolism for the efficient utilisation of its energy stores. In parasitic helminths, the end product of anaerobically functioning mitochondria is excreted in part as succinate and the remaining succinate is further metabolized to propionate which is also excreted. Föll et al. (1999) exposed a mixed population of *C. elegans* to anoxia and observed that the nematodes' metabolism was depressed to 3–4% that of the aerobic value. Enhanced excretion of lactate, acetate, succinate and propionate was detected during anoxia, consistent with the induction of the pyruvate fermentation and malate dismutation pathways in these nematodes. However, it seems untenable that dauer larvae would excrete energy rich metabolites such as acetate, propionate, succinate and lactate except under extremely adverse environmental conditions. It seems more likely that such metabolites would be transported to the peripheral tissues for aerobic metabolism and gluconeogenesis. Dauer larvae maintain a high degree of motility during the first 2–3 weeks after dauer formation. This motility declines with age, but older dauers become motile in response to mechanical stimulation. In muscles the gluconeogenetic pathway could be used to provide a store of readily accessible energy source in the form of glycogen, which could be metabolised to release single glucose units on demand. Ultrastructural studies show that *C. elegans* dauer larvae contain glycogen granules in neurons and in the muscles of the body wall and pharynx (Popham and Webster, 1979).

2.2. The generation of reactive oxygen species (ROS) by the mitochondrial electron transport chain

In aerobically functioning mitochondria, the electrons from NADH and $FMNH_2$ are transported to molecular oxygen by the proton pumping electron transport chain (Fig. 2). The resulting proton-motive force across the inner mitochondrial membrane is utilised by mitochondrial ATP synthase to phosphorylate ADP. Mitochondrial electron carriers transport a single electron. Molecular oxygen is capable of accepting a single electron from the mitochondrial electron transport chain to create a reactive superoxide ion. These reactive oxygen species (ROS) are generated mainly at complexes I and III of the respiratory chain (Raha and Robinson, 2000). Organisms are protected against ROS by superoxide dismutase (SOD), which converts superoxide ions to H_2O_2 which can be further deactivated by catalase, glutathione peroxidase or

peroxiredoxin. Dauer larvae have increased activities of SOD and catalase (Vanfleteren and De Vreese, 1996; Houthoofd et al., 2002). These observations have been confirmed in the SAGE and microarray experiments (Jones et al., 2001; Wang and Kim, 2003) and in addition, the SAGE experiment also detected a large increase in the expression of two glutathione peroxidase genes in dauer tags as compared with the mixed stages.

It might be expected that lowered aerobic metabolism in dauer larvae would reduce the production of ROS and this, combined with increased activity of SOD, catalase and glutathione peroxidase would protect dauer larvae from oxidative damage and contribute to dauer longevity. Interestingly, it was found that *E. coli* QFR (unlike SQR) produces substantial amounts of superoxide and hydrogen peroxide (Messner et al., 2002), while oxidizing succinate under aerobic conditions, and this was ascribed to a much higher electron distribution at FAD in QFR (Yankovskaya et al., 2003). Thus it is possible that the increases of SOD and catalase activities measured in dauer tissue may have evolved as a compensating protective adaptation associated with the utilisation of a mitochondrial complex II fumarate reductase when O_2 is limited.

3. Stress resistance, cellular maintenance and detoxification pathways are upregulated in dauer larvae

In addition to the increased activity of anti-ROS defence systems described above, dauer larvae also show increased transcriptional activity of other cellular defence systems. Transcripts encoding molecular chaperones are highly expressed in dauers. The most abundant of these was *hsp-20* (which encodes a member of the HSP-12.6 α-crystallin family of small heat shock proteins), but *hsp-70* and *daf-21* (a member of the HSP-90 family of heat shock proteins) were also upregulated. The α-crystallins are versatile protective agents variously involved in protein and membrane protection, as well as stabilisation of nucleic acids. HSP-20 is also expressed in populations of L1 larvae arrested by starvation but it was not detected in adults or other larval stages, nor it was induced in response to environmental or chemical stress (Leroux et al., 1997). Diapausing larvae of the brine shrimp *Artemia franciscana* which are capable of profound metabolic dormancy and extreme stress resistance also contain large amounts of an α-crystallin small heat shock protein (Liang et al., 1997). Taken together these data suggest that the accumulation of α-crystallin HSPs may be a specific response to developmental arrest in *C. elegans* and *A. franciscana*. Other highly expressed dauer transcripts may be involved in the stabilisation of nucleic acids and chromosomes. The most abundant tag in the dauer SAGE library (4329 dauer vs 215 in the mixed stage) was for a non-protein coding RNA named *tts-1* (transcribed telomere-like sequence). The function of *tts-1* is unknown, but Jones et al. (2001) postulate that it may have a chromosome protective function, possibly involving binding to the telomeres or telomere-associated proteins. Two histone H1-like genes showed increased dauer expression and the expression of one histone H1-like gene was reduced, suggesting that dauer chromatin may be altered. The DNA repair endonuclease C47D12.8 (Jones et al., 2001) and the protein repair gene *pcm-1* are also upregulated in dauer larvae (Kagan and Clarke, 1995), providing further evidence for the increased emphasis on maintenance and repair processes during dauer diapause.

Several broad spectrum detoxification gene families are also upregulated in dauer larvae. These include cytochrome P450-dependent monoxygenases (CYPs), and UDP-glucuronosyltransferases (UGTs) (Wang and Kim, 2003) as well as short-chain dehydrogenases/reductases (SDRs) (McElwee et al., 2004) and glutathione S-transferases (GSTs) (Jones et al., 2001). CYPs are involved in the biosynthesis or biodegradation of a range of endogenous lipophilic substrates such as steroids, fatty acids and aromatic compounds and also in the biodegradation of xenobiotics. SDRs also function in the metabolism of steroids, alcohols, lipophilic hormones and xenobiotics, typically catalysing the reduction of carbonyl groups in aldehydes and ketones. UGTs add a glucuronic acid group to a wide range of structurally diverse lipophilic molecules, thereby increasing their aqueous solubility, allowing them to be partitioned into intra- and extracellular aqueous compartments. GSTs catalyse the detoxification of a wide variety of endobiotic and xenobiotic electrophilic substrates by conjugation with glutathione, rendering the glutathione adducts less toxic and more water-soluble. Additionally, GSTs play a role in oxidative stress resistance. These detoxification reactions are metabolically costly, the CYP and SDR reactions consuming energy from NADH or NADPH, while each UGT and GST reaction consumes, respectively, a molecule of glucose or a molecule of glutathione. Since dauer larvae are non-feeding and are protected by a sealed intestine and resistant cuticle, these detoxification reactions are likely to be targeted towards degrading or metabolising endogenous compounds. Some of these reactions (e.g. CYP) may be involved in the biosynthesis or degradation of lipophilic hormones required for the maintenance of, or recovery from, the dauer state, but many of the compounds that this system targets are likely to be potentially toxic endogenous metabolites.

4. Some dauer-specific processes are reiterated in long lived *daf-2* adult nematodes

Genome-wide surveys of transcriptional changes resulting from reduced signalling through the DAF-2 insulin/IGF-1 receptor in long-lived *daf-2* adult nematodes have been carried out using microarray (McElwee et al., 2003, 2004; Murphy et al., 2003) and SAGE (Halaschek-Weiner et al., 2005) analyses. Although there is some overlap between the gene sets identified in the different experiments, a high proportion of genes is uniquely identified in each study. McElwee et al. (2004) identified a number of gene categories that significantly enriched in both dauer larvae and *daf-2* adult nematodes. These include the HSP20/α-crystallin gene family; detoxification genes encoding CYPs, SDRs and UGTs; genes encoding

transthyretin-related proteins (TRP), as well as other gene classes involved in metabolism, oxidoreductase activity and electron transport. Murphy et al. (2003) also found that genes involved in stress resistance and detoxification pathways are upregulated in *daf-2* adult worms (among them members of the HSP20/α-crystallin gene family). In addition, these authors found that several genes encoding anti-microbial proteins were upregulated in *daf-2* adults. The most prominent gene class detected in the SAGE analysis was also the HSP20/α-crystallin gene family, underlining the importance of this gene class in both dauer diapause and adult longevity. The differential expression of histone genes (McElwee et al., 2004) and of transcribed telomere-like sequence *tts-1* gene (Halaschek-Weiner et al., 2005) in *daf-2* adults suggests that the chromatin architecture of long-lived worms may also be modified, as was also postulated for dauer larvae (Jones et al., 2001).

Houthoofd et al. (2005) found that the O_2 consumption of *daf-2* adults was maintained at a higher level than controls from day 3 of adulthood onwards. However, *daf-2* adults dissipated less heat per unit of O_2 consumed and had elevated levels of ATP compared to wild type worms. Possible explanations proposed for these observations were that the contribution of anaerobic metabolism was small in *daf-2* adults, that respiration is more tightly coupled in *daf-2* adults, and that the balance between ATP production and consumption is altered in these animals, perhaps reflecting reduced anabolic rates. SAGE analysis revealed that several major metabolic systems involved in biosynthetic and catabolic activities (e.g. turnover of proteins and lipids, kinases, phosphatases, cell cycle regulation, synthesis of yolk proteins) showed reduced levels of expression in *daf-2* adults (Halaschek-Weiner et al., 2005). Transcripts related to carbohydrate metabolism (glycolysis and gluconeogenesis) and energy metabolism (electron transport and ATP synthesis) were also expressed at lower levels in long-lived *daf-2* adults than in controls. However, these SAGE analyses also found that *daf-2* adults do not share the anaerobic carbohydrate metabolism described in dauer larvae by Holt and Riddle (2003).

5. Conclusions and future prospects

Expression profiling has demonstrated that *C. elegans* dauer larvae and *daf-2* adults share a common life maintenance system which requires the co-ordinated expression of a large number of genes. Important components of this enhanced longevity module are the α-crystallin family of small heat shock proteins, anti-ROS defence systems, increased activity of cellular detoxification processes and possibly also increased chromatin stability and decreased protein turnover. Anaerobic fermentation pathways are upregulated in dauer larvae, while long lived *daf-2* adults appear have normal oxidative metabolism. Anabolic pathways are downregulated in both systems, possibly because of the expression of enhanced cellular maintenance and detoxification processes. Gems and McElwee (2005) propose that the energetically costly detoxification and enhanced cell maintenance processes are the key elements which have been selected in the dauer longevity programme. The longevity phenotype resulting from the ectopic expression of this dauer somatic maintenance programme in *daf-2* adult worms is in accord with the disposable soma theory of ageing (Kirkwood, 1977). This theory predicts that evolutionary fitness is optimised by investment in somatic maintenance only the minimum resources required to ensure successful reproduction, thereby restricting post-reproductive life-span.

Expression profiling studies have provided an important framework for further experimentation on dauer metabolism. Dauer larvae are a non-feeding system, so it should be possible to investigate metabolic network topologies and quantify metabolic fluxes through these pathways, yielding further insights into dauer physiology and longevity.

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