REVIEW/REVISIÓN

DAUER IN NEMATODES AS A WAY TO PERSIST OR OBVIATE

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ABSTRACT

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Dauer is a German word for "enduring" or "persisting". Dauer is an alternative larval stage in which development is arrested in response to environmental or hormonal cues in some nematodes such as *Caenorhabditis elegans*. At end of the first and beginning of the second larval stage, the animal may enter a quiescent state of diapause called dauer if the environmental conditions are not favorable for further growth. The dauer is a non-aging state that does not affect postdauer life span. Entry into dauer is regulated by different signaling pathways, including transforming growth factor, cyclic guanosine monophosphate, hormonal signaling pathways, and insulin-like signaling. The mechanistic basis for the effect of genetic or environmental cues on dauer arrest is similar to that of many persistent pathogens. Many outstanding questions remain concerning dauer biology, a fertile field of study both as a model for regulatory mechanisms governing morphological change during organismal development, and as a parallel to obligate dauer-like developmental stages in other organisms. Future research may lead to more powerful tools to understand the roles of those families detected in dauer arrest and could elucidate early cues inducing dauer formation.

Key words: Caenorhabditis elegans, cancer dormancy, dauer, developmental arrest, life span.

RÉSUMÉ

Wang, Y., and, X. Hou. 2015. Les juvéniles de stade "dauer larvae" chez les nématodes : échappatoire ou moyen de persister ? Nematropica 45:128-137.

Dauer est un mot d'origine germanique qui signifie "endurant" ou "persistant". Le stade "dauer larvae" est un stade juvénile alternatif chez certains nématodes comme *Caenorhabditis elegans*, ou le développement est interrompu suite à des signaux environnementaux ou hormonaux. A la fin du premier stade juvénile et au commencement du second, les individus peuvent entrer dans une période de repos ou diapause appelé "dauer larvae" si les conditions environnementales ne sont plus favorables au développement. Le stade "dauer larvae" est un stade de repos qui n'affecte pas la durée du futur cycle biologique. Le déclenchement du stade "dauer larvae" est régulé par différents signaux qui comprennent des facteurs de croissance, le cycle de la guanosine monophosphate, des signaux hormonaux et un signal proche de l'insuline. Le mécanisme de base de l'effet des clés génétiques ou environnementales sur l'arrêt du stade "dauer larvae" est semblable à celui de nombreux autres agents pathogènes persistants. Cependant, beaucoup de questions demeurent en suspens concernant la biologie de la "dauer larvae". Il s'agit là d'un futur domaine d'investigation à la fois comme modèle de mécanisme de régulation des changements morphologiques au cours du développement des organismes et comme un parallèle pour les stades de développement obligatoire de type "dauer larvae" chez d'autres organismes. Les recherches futures pourraient conduire à des outils plus puissants pour comprendre les rôles de ces organismes en arrêt de développement et pourraient aider à découvrir les premiers indices conduisant à la formation de ces "dauer larvae".

Mots clés: Caenorhabditis elegans, dormance du cancer, dauer, arrêt du développement, durée de vie.

INTRODUCTION

Caenorhabditis elegans is a prime candidate for addressing questions of gene regulation in a multicellular organism setting. Its sequenced genome, fully determined cell lineage, facile genetics, and well-studied developmental processes, represent a major model system in biology. In response to food depletion or overcrowding, this soil borne nematode can arrest development and enter an alternative larval stage, known as the dauer stage, which is a stress-resistant stage in response to unfavorable environmental conditions. In 1975, Cassada and Russell (1975) described an arrested developmental variant of *C. elegans* that forms at the second molt in response to environmental duress. Dauer larvae are easily distinguished from other developmental stages. They are thin and dense due to shrinkage of the hypodermis at the dauer-specific molt (Cassada and Russell 1975; Ruzanov *et al.*, 2007). At end of the first larval stage (L1), the animal may enter this quiescent state of diapause if the environmental conditions (which may include the presence of a pheromone, high population density, limited food, or increased temperature) are not favorable for further growth. These normal dauers are characterized by markedly reduced locomotion, and the nematodes are very thin with a thick, altered cuticle. The buccal cavity is sealed by a cuticular block, the pharyngeal and intestinal lumens are shrunken, small and indistinct microvilli are found in the intestine, and the excretory gland lacks secretory granules.

Dauer larvae were first identified as a special larval stage of insect-parasitic nematodes (Bovien, 1937). Well-fed worms live for about 3 wk, but dauer larvae can live for at least 2 mon without affecting post-dauer lifespan. Consequently, the dauer is a non-aging diapause and an alternative larval stage capable of long-term survival, similar to hibernation in mammals. Dauer formation in *C. elegans* is a temperature-sensitive process controlled through a network of signaling pathways functioning as a genetic switch. The environmental cues are integrated throughout the L1 stage and the primary cue is a *Caenorhabditis*-specific pheromone, which is developmentally regulated (Butcher *et al.*, 2008; Joo *et al.*, 2010; Lee *et al.*, 2010; Kaplan *et al.*, 2011). Both cellular and genetic experiments have revealed redundant or overlapping neural functions that may govern whether or not the larva enters the dauer stage. Although research on the *C. elegans* dauer larva has been reviewed elsewhere (Ludewig and Schroeder, 2013), the focus of the review was on genetics and biochemistry (Wang *et al.*, 2009). This review will focus on dauer larvae environmental ecology.

The regulatory plasticity of dauer formation

The dauer is a developmental stage in *C. elegans* that exhibits increased longevity, stress resistance, nictation, and altered metabolism compared with normal worms. Gene expression in a developmentally arrested, long-lived dauer population of *C. elegans* was compared with a nondauer (mixed-stage) population by using serial analysis of gene expression (SAGE) (Baillie *et al.*, 2001). Dauer (152,314) and nondauer (148,324) SAGE tags identified 11,130 of the predicted 19,100 *C. elegans* genes. A total of 2,618 genes were detected in the nondauer population, whereas 2,016 genes were detected in the dauer, showing that dauer larvae have a surprisingly complex gene expression profile. Evidence for differentially expressed gene transcript isoforms was obtained for 162 genes. Ruzanov *et al.* (2007) also used SAGE to compare the global transcription profiles of long-lived mutant daf-2 adults and dauer larvae, aiming to identify aging-related genes based on similarity of expression patterns. Comparison of eight SAGE libraries yielded a set of 120 genes, which may modulate longevity in *C. elegans* in both dauer larvae and long-lived daf-2 adults. Oh *et al.* (2006) used chromatin immunoprecipitation (ChIP) to clone 103 target sequences containing consensus DAF-16 binding sites and selected 33 targets for further analysis.

Wang and Kim (2003) have used DNA microarrays to profile gene expression differences during the transition from the dauer state to the nondauer state after feeding starved L1 animals. They identified 1,984 genes that show significant expression changes. This analysis included both genes that encode transcription factors and components of signaling pathways that could regulate the entry to and exit from the dauer state, and genes that encode components of metabolic pathways important for dauer survival and longevity. In the dauer profile, a relatively greater proportion of highly abundant transcripts was counterbalanced by a smaller fraction of low to moderately abundant transcripts. Comparisons of abundant tag counts between the two profiles revealed relative enrichment in the dauer profile of transcripts with predicted or known involvement in ribosome biogenesis and protein synthesis, membrane transport, and immune responses. Translation-coupled mRNA decay was proposed as part of an immune-like stress response in the dauer larva.

Elling *et al.* (2007) conducted a comprehensive analysis of large-scale gene expression changes throughout the development of plant-parasitic nematodes beginning with the generation of 20,100

expressed sequence tags (ESTs). They suggested divergent evolution of arrested development in the dauer stage of *C. elegans* and the infective stage of *Heterodera glycines*, and showed that the arrested development in the *C. elegans* dauer larva and the *H. glycines* infective second-stage juvenile (J2) exhibited shared gene expression profiles. Entry into the dauer is regulated by different signaling pathways, including transforming growth factor (TGF), cyclic guanosine monophosphate, hormonal signaling pathways, and insulin-like signaling (ILS). Heat shock factor (HSF) and molecular chaperones act in multiple tissues to regulate development and longevity (Morley and Morimoto, 2004).

Ailion and Thomas (2003) isolated and characterized high-temperature-induced dauer formation mutants in *C. elegans*. Heat shock factor regulates the expression of genes involved in growth under normal physiological conditions (Wang *et al.*, 2007). Low temperatures can assure the long-term or even indefinite preservation of important biological specimens. Heat shock factor functions at the convergence of the stress response and developmental pathways in *C. elegans*, and the ability to integrate the stress response with development may be an essential element of its ecology. In an ILS mutant, hsf-1 is required for temperature-induced dauer larvae formation (Ailion and Thomas, 2003). Jones *et al.* (2010) found six genes that were three- to nine-fold upregulated in dauer larvae. After correction for mRNA load, genes encoded poly (A)-binding protein (PABP), heat-shock proteins hsp70 and hsp90, and three novel genes of uncertain function were identified. Diverse *C. elegans* genes that are upregulated in dauer larvae also show elevated transcript levels in long-lived, aged, or starved adults. The interaction of ILS with HSF-1 could represent an important molecular strategy to couple the regulation of longevity with an ancient genetic switch that governs the ability of cells to sense and respond to stress.

Dauer formation pathways impact growth, metabolism, survival, and aging

Dauers can survive several times longer than the normal life span and the duration of the dauer state has no effect on postdauer life span. Questions of whether this non-aging attribute may lead to eventual mortality as a consequence of depletion of stored nutrients have been raised. A second question is whether senescence in dauer larvae that occurs before attainment of reproductive maturity is reversible. The L1 diapause that is exhibited by some phenotypes of *C. elegans* is similar to the dauer stage, although appears to be related to certain aging genes (Baugh and Sternberg, 2006). Consistent with its role in dauer formation and aging, insulin/insulin-like growth factor (IGF) signaling regulates L1 arrest, which is more resistant to environmental stress than developing larvae (Hoogewijs *et al.*, 2008). *Caenorhabditis elegans* mutants that experience caloric restriction because they are feeding-defective exhibit decreased levels of fat deposits as well as smaller body size. The dauer larva slowly develops senescence-like symptoms including a decrease in metabolic capacity and ATP stores, and an increase in lipofuscin- and oxidised flavin-specific fluorescence. However, these changes and other life processes, including respiration rate and heat output, are reversed when the dauers recover.

Dauer larvae do not need to feed to live; their metabolism is dependent on internal food reserves. The ability of dauer larvae to live several times longer than individuals that undergo normal, continuous developmental life has been attributed, in part, to a repressed metabolism (Wang *et al.*, 2009). However, the molecular pathways that link nutritional cues to developmental programs are poorly understood. Generation of nutritive fermentation byproducts and the moderation of oxidative damage are potential benefits of a hypoxic dauer interior. Autophagy, associated with formation of the dauer larva, is a catabolic process in which long-lived proteins and organelles are degraded for recycling in the cytoplasm. Fukuyama *et al.* (2006) suggested that caloric restriction may increase the expression of FKHR-family (mammalian daf-16 homologues) genes and prevent the aging process in skeletal muscles. Metabolic and transcription rates are lowered but the transcriptome of the dauer is complex. In the dauer profile generated by SAGE, a relatively greater proportion of highly abundant transcripts were counterbalanced by a smaller fraction of low to moderately abundant transcripts (Green *et al.*, 2013).

The dauer larva is long-lived and stress resistant. Dauer-inducing pheromones, including daumone also extend the adult lifespan in *C. elegans* (Jeong *et al.*, 2005; Butcher *et al.*, 2007, 2008, 2009). Metabolic stress has severe health consequences including accelerated aging (Epel, 2009). The correlation between longevity and stress resistance suggests that the ability to sense to environmental challenges could be important for the regulation of life span by heat shock factor and molecular chaperones. Information on similarities and differences in metabolism, which may be elucidated by more thorough study of dauer regulation of the genes relevant to steroid and xenobiotic metabolism, may provide greater insight. This phenomenon appears to correspond to dauer formation in *C. elegans*, and many dauer formation (Daf) mutants affect longevity and stress resistance

(Wang *et al.*, 2013, 2014). Genes of the dauer/insulin/insulin-like signaling (IIS) pathway appear to have well-established roles in aging in *C. elegans* (Tissenbaum and Guarente, 2001).

Environmental and molecular cues are coupled to evolutionarily conserved pathways

The developmental response to environmental conditions is an example of phenotypic plasticity and a manifestation of a genotype by environment interaction. Extensive variation was found in reaction norms of phenotypic plasticity of dauer formation among wild lines of *C. elegans* (Sommer and Ogawa, 2011; Okumura *et al.*, 2013) The natural variation in reaction norms of different lines in dauer formation in *C. elegans* is presumably an adaptation to enhance fitness under different natural prevailing conditions (Okumura *et al.*, 2013). Despite low worldwide diversity among natural populations of *C. elegans*, local populations are genetically diverse and a low frequency of outcrossing allows for the recombination of these locally diverse genotypes, probably owing to transient bottlenecks and ongoing dispersal as a dauer larva (Sommer and Ogawa, 2011).

Caenorhabditis elegans is found predominantly in the dauer stage with a very low frequency of males relative to the number of hermaphrodites. The main mode of reproduction in *C. elegans* population is likely selfing, which predominates in the wild, although rare outcrossing may also play a role in the population development (Tang and Wang, 2012). In both females and males, the development of somatic gonads generally begins in the first larval stage, whereas in hermaphrodites gonad development is delayed until the second larval stage. Vulval development also differs between females and hermaphrodites (Félix, 2004). The dauer state, while allowing for survival under adverse conditions, replaces the stage of normal development that is critical for the reproductive organs and has important developmental and reproductive consequences. The seam cell is essential for the structural integrity of adult hermaphrodites in the vulval region and for diametric shrinkage during dauer larval formation. In *C. elegans*, population density is monitored through the dideoxysugar ascarylose glycoside (the 'ascarosides'), which promotes entry into the dauer stage. Adult males are attracted to hermaphrodites by a small-molecule signal that consists of a synergistic blend of three dauer-inducing ascarosides (Srinivasan *et al.*, 2008). The common set of signaling molecules named ascaroside connects reproductive and developmental pathways. It is interesting that the ascarosides act as a potent male attractant at very low concentrations, whereas at the higher concentrations required for dauer formation the compounds no longer attract males and instead deter hermaphrodites. Kim and Paik (2008) report that increased duration of diapause causes a delay in post-dauer development, and also causes severe defects in the reproductive development of males and hermaphrodites. This effect is more pronounced in males, possibly accounting for the increased survival of *C. elegans* hermaphrodites under challenging environmental conditions.

In *C. elegans*, reduced insulin-like signaling induces developmental quiescence and reproductive delay (Dumas *et al.*, 2013). Reduced TGF-β activity also triggers developmental quiescence independent of the insulin-like pathway. In humans, germline mutations in TGF-β family members, PTEN or LKB1 result in related tumour-predisposing syndromes (Narbonne and Roy, 2009). Narbonne and Roy (2009) found that the inhibition of germline proliferation during the *C. elegans* dauer state requires PTEN and AMPK signaling. The inactivation of either protein causes aberrant germline proliferation in the dauer stage, whereas the loss of AMPK uncouples developmental arrest from lifespan extension. The two signaling pathways converge on the *C. elegans* PTEN orthologue to coordinate germline proliferation with somatic development during dauer formation, via the regulation of AMPK and its upstream activator LKB1, rather than through the canonical insulin-like signaling cascade (Hardie, 2011).

Dauer can be conveniently described and analyzed at the cell level

The basic cell cycle is like a comprehensive regulatory network that incorporates environmental factors and coordinates cell division, and affects many aspects of development. CKI-1, a Cyclin-Dependent Kinases inhibitor of the Cip/Kip family, is critical for the temporal control of cell division and dauer regulatory pathways. Loss of the DAF-18/PTEN tumor suppressor bypasses developmental arrest, resulting in inappropriate germline growth that is dependent on the AGE-1/PI-3 and AKT-1/PKB kinases. *Caenorhabditis elegans* hatchlings arrest in a dormant state termed L1 diapause. The embryonic germline precursors undergo G2 arrest with condensed chromosomes and remain arrested throughout L1 diapause. This is not a passive consequence of nutrient deprivation, but is actively maintained by DAF-18 through a pathway distinct from that which regulates longevity and dauer formation. DAF-16 is required for transcription of the cyclin-dependent kinase inhibitor cki-1 in stem cells in response to starvation. This accounts for the failure of daf-16/FOXO mutants to arrest cell division during

L1. DAF-16/FOXO promotes developmental arrest via transcriptional regulation of numerous target genes that control various aspects of development such as cell migration and cell fusion (Ogawa *et al.*, 2011). DAF-18/PTEN mediates nutrient-dependent arrest of the cell cycle and growth in the germline (Fukuyama *et al.*, 2006).

Although *C. elegans* integrates a variety of sensory information to commit to dauer formation, it is currently unknown whether they also monitor internal cellular rest or cell damage. Two neuron classes, ADF and ASI, control entry into the environmentally resistant resting and dispersal dauer larval stage. A daf-28GFP fusion gene is expressed in ASI and ASJ, two sensory neurons that regulate dauer arrest and control the developmental switch. The *C. elegans* che-1 gene encodes a zinc finger transcription factor required for specification of the ASE chemosensory neurons, which have a major role in the behavior of chemotaxis to water-soluble chemicals. Chemosensory cues can elicit chemotaxis, rapid avoidance, changes in overall motility, and entry into and exit from the dauer stage. These behaviors are regulated primarily by the amphid chemosensory organs, which contain eleven pairs of chemosensory neurons (Kulalert and Kim, 2013). Each amphid sensory neuron expresses a specific set of candidate receptor genes and detects a characteristic set of attractants, repellents, or pheromones. The chemosensory neurons and signaling pathways that control dauer recovery in *C. elegans* also control infective juvenile recovery in *Heterorhabditis*, suggesting conservation of these developmental processes across free-living and parasitic nematodes (Chaisson and Hallem, 2012).

Autophagy through the sequestration and delivery of cargo to the lysosomes is the major route for degrading cytoplasmic long-lived proteins and organelles in eukaryotic cells. Macrophage migration inhibitory factor (MIF), a molecule that exerts a wide-range of effects in inflammatory responses, cell activation, and cell differentiation in vertebrate species, plays a role in cellular maintenance in *C. elegans* during periods of adverse conditions that lead to developmental arrest (Marson *et al.*, 2001). Dauer formation is associated with increased autophagy and requires *C. elegans* orthologs of the yeast autophagy genes APG1, APG7, APG8, and AUT10. The genes required for autophagy act downstream of insulin-like signaling, and are involved in the expression of major life history traits, including dauer larva development and adult life span (Alberti *et al.*, 2010). Autophagy, which could be involved in the protection against apoptosis, is a protective mechanism in chronic ischemia (Carloni *et al.*, 2008).

The dauer shares similarities with the induction of autophagy in chronic myocardial ischemia and hibernating myocardium. Thus, autophagy is a cellular pathway essential for dauer formation and life-span extension in *C. elegans*, which is activated by environmental stresses and confers stress resistance to the organism. Novel relationships between caloric restriction, longevity, body size development, and autophagy may occur. Biological responses due to nutrient deprivation in *C. elegans*, including L1 diapause and autophagy during dauer formation, can be mediated through the linked DAF-2/insulin/IGF receptor and target-of-rapamycin (TOR) kinase pathways. Gomez et al (2007) found that a null mutation in the pcm-1 gene can inhibit autophagy during dauer formation and decreased L1 arrest survival, suggesting that the absence of protein repair may also interfere with protein degradation pathways. PCM-1 may function either directly or indirectly as an inhibitor of insulin/TOR signaling, perhaps in a role to balance autophagy with alternative protein degradation pathways.

Similar processes in other organ and homologue in disease states

Studies on dauer larvae are relevant not only to nematode biology but also to human health, as the evolutionary conservation of these signal transduction pathways suggests that what we learn about interactions during *C. elegans* larval development may be germane to the interactions of similar signaling pathways in the pathogenesis of common diseases such as diabetes mellitus and cancer. The human PTEN tumor suppressor gene is mutated in a wide variety of sporadic tumors. The PTEN tumor suppressor homolog in *C. elegans* regulates longevity and dauer formation in an insulin receptor-like signaling pathway (Mihaylova *et al.*, 1999). Analysis of dauer larva development in *C. elegans* by daf-18, a homologue of the tumor suppressor PTEN, should shed light on the role of human PTEN in the etiology of metabolic disease, aging, and cancer. Furthermore, PTEN have been identified that negatively regulate the insulin/IGF pathway in a whole organism and raise the hypothesis that PTEN may be involved in mammalian aging (Carracedo and Pandolfi, 2008). The human PTEN can substitute for DAF-18 and restores the dauer and longevity phenotypes in worms devoid of DAF-18. Hematopoietic stem cells (HSCs) reside in the bone marrow (BM) niche in a noncycling state and enter the cell cycle at long intervals. Lipid raft clustering induced by cytokines is essential for HSC re-entry into the cell cycle. The lipid rafts may play a critical role in regulating the cell cycle, the survival, and the entry into apoptosis of HSCs and uncover a striking

similarity in HSC hibernation and *C. elegans* dauer formation.

Some animals show arrested states that are similar to dauer stage of worms or reproductive diapause of some insects (McGrath *et al.*, 2011). Hibernation in mammals is a reversible state of suspended animation that is associated with tolerance to an otherwise lethal reduction of core body temperature and metabolism. Diapause is also a state of arrested development accompanied by somatic persistence. Diapause is common in many invertebrates and is familiar to biogerontology in the context of the *C. elegans* dauer. Among insects, diapause may occur in embryos, larvae, pupae, or adults. At the adult stage, reproductive diapause arrests development of oogenesis and vitellogenesis, accessory gland activity, and mating behavior. Reproductive diapause has been well studied in monarch butterflies, grasshoppers, and several *Diptera*.

Like dauer stage of *C. elegans*, arrest states of other animals could be very useful models for some diseases. An integral aspect of hibernation is tolerance to a profound decrease of cerebral perfusion. Identification of regulatory mechanisms that control hibernation in ground squirrels could guide efforts to develop improved treatment for stroke and brain trauma. Joshua *et al.* (2003) used a *C. elegans* model of Yersinia infection for biofilm formation on a biotic surface. They suggested that biofilm formation on a biotic surface is an interactive process involving both bacterial and invertebrate control mechanisms. Hallem *et al.* (2007) also developed a tripartite model for nematode parasitism of nematodes, bacteria, and flies. It is interesting to speculate whether the adaptive mechanisms that regulate cancer dormancy (or other biological behave similar to dormant) have any parallel with those regulating *C. elegans* dauer stage.

The roles of phosphatidylinositol 3-kinase (PI3-kinase) for both diapause in *D. melanogaster* and dauer formation in *C. elegans* suggest a conserved role for this kinase in both reproductive and developmental arrests in response to environmental stresses (Williams *et al.*, 2006). Natural variation in organs during diapause may be regulated by the same pathway or genes, for example, the insulin-regulated PI3-kinase. Biofilm formation on the biotic surface is an interactive process involving both bacterial dormancy and nematode dauer mechanisms. There may be a conservation of developmental processes across the dauer stage of *C. elegans* and persisters of *E. coli* (Wang *et al.*, 2009). Slow aging during the diapause period may involve elevated somatic stress resistance as well as reallocation of resources to somatic maintenance (Carracedo and Pandolfi, 2008). The neuroendocrine control of reproductive diapause includes phenotypic plasticity for rates of senescence. Reproductive diapause in *Drosophila* is proximally controlled by down regulation of juvenile hormone, a phenotype that is also produced by mutants of the insulin-like receptor, homologue of *C. elegans* daf-2 (Jones *et al.*, 2010). Akt is a key molecule in the insulin/insulin-like growth factor signal transduction pathway, which plays a critical role in the balance between survival and apoptosis. Dauer formation in *C. elegans* where Akt inhibition is associated with energy conservation, fat storage, expression of antioxidant enzymes, and growth arrest (Paradis and Ruvkun, 1998).

More than a quarter of the world's population is infected with nematode parasites, and more than a hundred species of nematodes are parasites of humans (Hallem *et al.*, 2007). In invading nematodes, only the dauer juvenile, the stage in the life cycle which is capable of surviving outside its host, can serve as an infective stage in the natural environment. In the evolution of animal parasitism, parasitic nematodes have taken signaling pathways and molecules from their free-living ancestors and used them in different ways in the evolution of their parasitic lifestyles. The ILS pathway and the TGF-β pathway, involved in regulating dauer larva formation in *C. elegans*, may influence the developmental timing and maturation in nematode parasites (Kiss *et al.*, 2009). Hallem *et al.* (2007) reported that the chemosensory neurons and signaling pathways that control dauer recovery in *C. elegans* also control dauer juvenile recovery in Heterorhabditis, suggesting conservation of these developmental processes across free-living and parasitic nematodes. The dauer stage of *C. elegans* is a developmentally arrested stage similar to that in the hookworm infective larva. The identification of an orthologue in *C. elegans* opens the way for further studies into the biological functions of helminth parasites. Understanding the differences in how these pathways are affected by environmental cues in free-living and parasitic species may provide insight into the mechanisms for the control of developmental arrest or response to environmental stress.

Culture and marker methods

Studies of *C. elegans* have almost exclusively utilized growth on a bacterial diet, and monoxenic cultivation of *C. elegans* on Nematode Growth Medium agar plates with *E. coli* (NGM) is standard. This method of culturing, however, presents a challenge to automation of experimentation and introduces bacterial metabolism as a secondary concern into drug and environmental toxicology studies. Axenic cultivation of *C. elegans* could eliminate these issues, but past work suggests that

axenic growth is unhealthy for *C. elegans*. Large scale screening of pharmaceutical and nematicidal compounds on *C. elegans* can now be achieved when liquid CeMM is used with equipment for automated culturing and experimentation. CeMM may also be useful in growing large numbers of animals for genomic or proteomic work and in development of *C. elegans* biosensors.

Cultivation in chemically defined media was promoted at the initial suggestion of the utility of *C. elegans* as a genetic model system. However, *C. elegans* cultured on some chemically defined liquid media was characterized by changes in gene expression as well as slower development, lower fecundity, decreased stores of lipid and protein, and an increased lifespan relative to individuals grown on a bacterial diet (Szewczyk *et al.*, 2006). Development and reproductive period are fixed percentages of the nematode lifespan regardless of diet, suggesting that these alterations are adaptive. The chemically defined liquid medium is a powerful system for automation of experimentation on healthy *C. elegans* and for systematic analysis of the impact of diet on animal physiology. Gomez *et al.* (2007) found that pcm-1 mutant L1 larvae do not survive as well as wild-type L1 larvae when incubated in M9 medium without nutrients. When L1 larvae were starved in cholesterol-containing S medium or M9 medium supplemented with cholesterol, the survival rates of both mutant and wild-type animals nearly doubled, with pcm-1 larvae faring more poorly than N2 larvae.

Caenorhabditis elegans diapause, gonadal outgrowth, and life span are regulated by a lipophilic hormone, which serves as a ligand to the nuclear hormone receptor DAF-12 (Dong *et al.*, 2007). Better understanding of the fundamental mechanisms behind metabolic diseases requires methods to monitor lipid stores on single-cell level in vivo. Hellerer *et al.* (2007) using spectral coherent anti-Stokes Raman scattering (CARS) microscopy measurements, indicated that this is accompanied by a shift in the ordering of the lipids from gel to liquid phase. This suggests a potential to become a sensitive and important tool for studies of lipid storage mechanisms, improving our understanding of phenomena underlying metabolic changes in dauers. Some studies have shown that glcyosides called ascarosides promote entry into the non-feeding and highly persistent dauer stage (Jeong *et al.*, 2005; Butcher *et al.*, 2007; 2008; 2009). The alae, longitudinal ridges of the lateral cuticle, are present only in L1 and dauer larvae and in adults. CeCYP-16::GFP-expressing lines have been generated with expression in the anterior and posterior distal portions of the intestine in all larval stages and adults except in dauer, where fluorescence was observed in both the cell bodies and processes of the ventral chord motor neurons but was absent from the intestine. These specific patterns of localization could be the marker for the dauer. Small-scale cultures for experimental purposes may be undertaken using minor modifications of standard *C. elegans* methods. Morphological similarities between *C. elegans* and the free-living stages of the threadworm, *Strongyloides stercoralis* allow investigational methods such as laser cell ablation and DNA transformation by gonadal microinjection to be easily adapted from *C. elegans* to other organs. Comparative studies employing these methods have yielded new insights into the regulation of dauer development in *C. elegans*.

Daumone: A pheromone or a cue?

Pheromones are cell type-specific signals used for communication between individuals of the same species. When faced with overcrowding or starvation, *C. elegans* secrete a small-molecule signal, traditionally called the dauer pheromone or the pheromone daumone, which facilitates communication between individuals for adaptation to adverse environmental stimuli (McGrath *et al.*, 2011). Daumone is an indicator of population density and influences pathways that regulate metabolism, development, and aging. It signals *C. elegans* to enter the dauer stage. Because daumone is a key regulator of chemosensory processes in development and aging, the chemical identification of daumone is important for elucidating features of the daumone-mediated signaling pathway. Jeong *et al.* (2005) isolated natural daumone from *C. elegans* by large-scale purification, and estimated the total chemical synthesis of the pheromone. The stereospecific chemical structure of purified daumone, a fatty acid derivative, was suggested and they demonstrated that both natural and chemically synthesized daumones induce dauer larva formation in the N2 strain of *C. elegans* and certain dauer mutants equally well.

Crude dauer-inducing pheromone extract could extend the adult lifespan in the animal. This extension does not occur in the mutant animal, in which expansion of the lifespan caused by other mutations reducing insulin signaling is suppressed. Preliminary fractionation of the lipophilic extracts shows that the activity is hydrophobic with some polar properties, consistent with a small lipophilic hormone (Monje *et al.*, 2011). Butcher *et al.* (2007) showed that the dauer pheromone consists of several structurally related ascaroside derivatives of the dideoxysugar ascarylose, and that two of these ascarosides are roughly two orders of magnitude more potent at inducing dauer formation and constitute a physiologically relevant signal. There are

also some small-molecule pheromones that control dauer development in *C. elegans*. *Caenorhabditis elegans* mutants that disrupt the function of sensory neurons required for the action of the previously characterized dauer pheromone blocked pheromone-induced resistance to halothane (Sommer and Ogawa, 2011). It has been proposed that lipophilic hormones act downstream of these pathways to regulate dauer formation (Butcher *et al.*, 2009). The difference between a signal (e.g., a pheromone) and a cue (e.g., a waste product) is that the information content of a signal is subject to natural selection, whereas that of a cue is not. There is some question as to whether dauer pheromone of *C. elegans* is really a pheromone (Jeong *et al.*, 2005; Butcher *et al.*, 2007). There is no doubt, however, that the model free-living nematode *C. elegans* forms the dauer larva in response to daumone, which is produced by all worms, although this is not a fitness advantage for an individual (Joo *et al.*, 2010).

*Prospec*t

Over the past decade, much has been learned about the molecular and cellular underpinnings of the regulation of dauer arrest. It has been commonly thought that the dauer state is a non-aging state. Several questions remain. For example, do dauer larva senesce? Are the dauer larva really in reproductive diapause? Significant progress has been made in defining the components of signal transduction pathways that regulate dauer formation. Other critical questions that remain unanswered include the molecular identity of the food signal, how it is interpreted by the animal, and how changes in ambient temperature are translated into molecular events that influence dauer arrest. It is likely that these environmental signals will function primarily by modulating signaling flux through the guanylyl cyclase, TGF-β-like, insulin-like, and hormonal pathways. The interface among signal transduction pathways that regulate dauer arrest will continue to be an active area of investigation. Although epistasis analysis has contributed substantially to our understanding of how these signaling pathways interact, further studies are certain to yield greater insight into the complexities of their interactions. The degree of hypodermal lipid storage and the lipid phase can be used as a marker of lipid metabolism shift, as the growth requires a metabolic tradeoff. The availability of synthetic dauer pheromone components should facilitate the search for specific pheromone receptors. The dauer nematodes and dormancy cells would link between organism and cell. However, many outstanding questions remain in the field. Future research should identify roles for other genetic or environmental cues.

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