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— INVITED REVIEW —

Diapause: diverse states of developmental and metabolic arrest

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Diapause, characterized by developmental arrest and metabolic inhibition, is a widespread phenomenon, occurring either in response to environmental changes or as genetically predetermined life history stages. Organisms undergoing diapause experience increased longevity, coordinated reproduction, and enhanced stress resistance, adaptive traits that promote survival under harsh conditions often not conducive to life. Diapause is studied from several perspectives, such as identification of regulatory cues, detection of hormonal signals appearing in response to external influences, differential gene expression, and gene flow within populations. Signaling pathways regulating environmentally induced dauer in *Caenorhabditis elegans* have been studied extensively and are described in the review. Hormonal control of gene expression during insect diapause, although less well understood at molecular/biochemical levels than *C. elegans* dauer, is considered, as is the remarkable stress resistance exhibited by encysted *Artemia* embryos during and after diapause, and the potential role of a small heat shock protein in the process. A profile of delayed implantation in certain mammalian embryos completes the review. Attention is given to the recent literature and to experimental advantages offered by divergent model systems as they relate to particular aspects of diapause.

Key words: diapause, quiescence, physiological stress, molecular chaperones, gene regulation, intracellular signaling, *Caenorhabditis* dauer, delayed embryo implantation, insects, *Artemia franciscana.*

Diapause, a condition of developmental and metabolic arrest exhibited by animals ranging from nematodes to mammals, is accompanied by physiological changes that enhance stress survival and synchronize reproduction (Hairston, 1998; Renfree & Shaw, 2000; Denlinger, 2001, 2002; MacRae, 2001, 2003; Hamatani *et al*., 2004). Diapause is characterized by mechanistic variations from one organism to another, although there are also shared properties, and the process occurs either in response to environmental conditions or during genetically determined life history stages. In the latter case diapause initiates prior to adversity and endures, even in favorable circumstances, until activation of dormant organisms. Behavior is altered during diapause and several activities are displayed; environmental cues are monitored, information is stored until the appropriate time for use, metabolism declines, cell growth and proliferation arrest, development stops, the body is maintained and growth eventually resumes (Denlinger, 2002).

Many important questions concerning diapause remain unanswered. What are the molecular mechanisms leading to cell cycle and growth arrest (developmental diapause) and to inhibition of metabolism (metabolic diapause), and are these similar from species to species? Stated in another way, how are specific genes regulated so that behavioral patterns, metabolism, and protein synthesis are modified, thereby leading to the production of storage proteins, extracellular coverings and protective molecules necessary in adverse settings? One possibility is a hormonal signal of maternal origin occasioned

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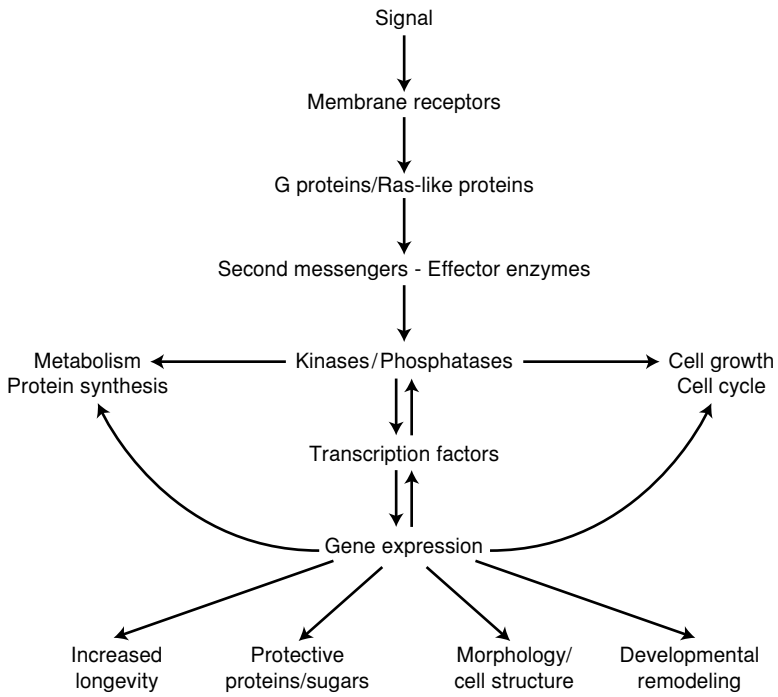

FIG. 1. A generalized mechanism able to promote and maintain diapause. Information flow originates from signals produced in response to environmental cues such as day length and temperature. The signal interacts with membrane receptors, initiating a cascade of responses involving second messengers, effector enzymes, kinases and phosphatases. Protein synthesis, metabolism, cell growth, cell cycle and gene expression may either be promoted or inhibited. Consequently, numerous cell and organismal properties are modified, resulting in diapause initiation and maintenance.

by environmental cues and leading to a continuous program of events. A single molecular mechanism, either sequentially or coordinately controlling transcription, translation and metabolism may operate in diapause-destined organisms, establishing a regulatory scenario with the potential to promote synchrony of interdependent processes responsible for diapause maintenance. Although there is limited appreciation of the physiological mechanisms underpinning diapause, models can be proposed and tested, these based heavily on the molecular dissection of *Caenorhabditis elegans* dauer. Information contained in signals recognized by membrane receptors is passed by way of second messengers to kinases and associated regulators responsible for modulation of cell division and a host of other downstream processes which dictate whether organisms continue growth and development or enter diapause (Fig. 1).

CAENORHABDITIS ELEGANS DAUER

Dauer regulation by interdependent insulin/IGF and TGFβ pathways

When food is plentiful *C. elegans* pass rapidly through four larval stages and become short-lived reproductive adults. In contrast, larvae may enter dauer (diapause), a condition of arrested development induced by starvation and crowding, and which reverses when food is supplied. Unlike types of diapause to be described later, *C. elegans* dauer happens in response to and not in anticipation of adversity, however it entails developmental remodeling, metabolic repression, enhanced stress resistance and increased longevity, all observed in other diapause organisms. The analysis of *daf* (dauer formation) mutants reveals that dauer metamorphosis is regulated by parallel genetic pathways influencing different mechanistic parameters (Fig. 2). In the insulin/insulin-like growth factor pathway (insulin/IGF), mutations resulting in the loss of *daf-2* products, a gene encoding an insulin receptor family member with tyrosine kinase activity, halt development at dauer and show increased longevity, as is true for mutants of *age-1*, a gene encoding the phosphatidylinositol-3-kinase catalytic subunit (Kimura *et al*., 1997; Tissenbaum & Ruvkun, 1998). The activity of *age-1* encoded kinase is antagonized by a downstream homologue of the mammalian *PTEN* tumor suppressor gene, *daf-18*, which encodes a phosphatidylinositol 3,4,5-trisphosphate (PIP3) phosphatase that ensures transient activation of the DAF-2 pathway (Gil *et al*., 1999; Rouault *et al*., 1999). Loss of function mutations to *daf-2* and *age*-1 leading to dauer are suppressed by *daf-18* loss of function mutations. The *daf-16* mutations also negate or reduce the effects of

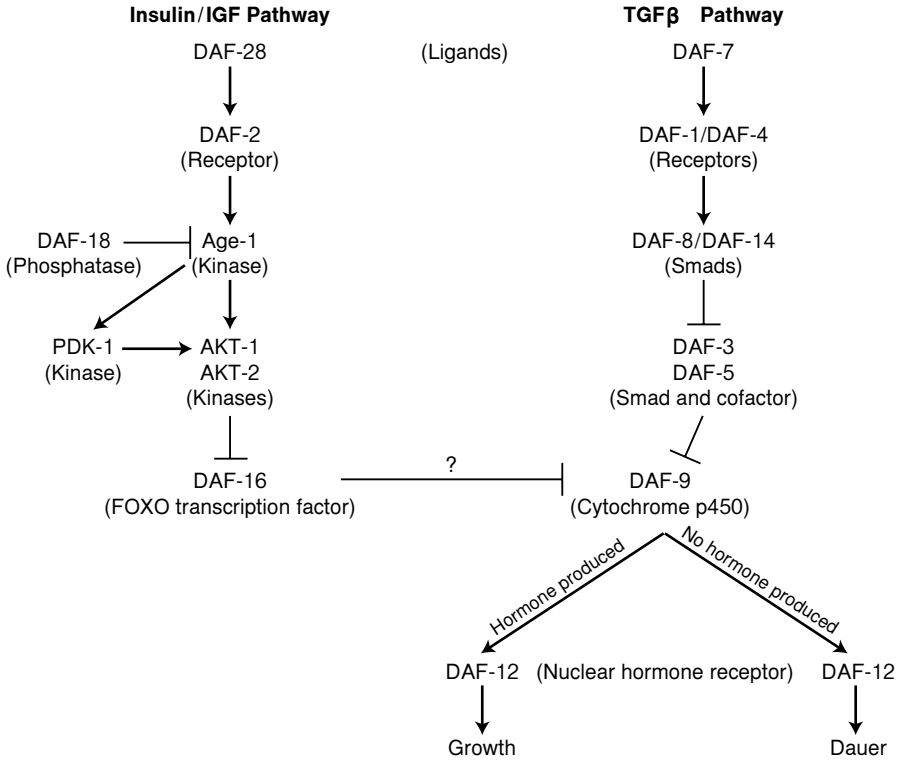

FIG. 2. *C. elegans* growth and dauer are regulated by the interplay of insulin/IGF and TGFβ signaling pathways. Details of the insulin/IGF and TGFβ pathways, which determine if *C. elegans* larvae develop into reproductive adults or enter dauer, are described in the text. The pathways are shown tentatively to merge at DAF-9/DAF-12, but this remains under investigation. Adapted from Liu T *et al*. (2004) and Ludewig *et al*. (2004).

daf-2 and *age-1* mutations, yielding dauer-defective phenotypes (Tissenbaum & Ruvkum, 1998). These and other genetic analyses indicate the insulin pathway modulates growth and dauer by negative regulation of DAF-16, a Forkhead type O (FOXO) transcription factor able to enter nuclei when dephosphorylated under adverse conditions and then promote longevity, stress resistance and dauer (Jones *et al*., 2001; da Graca *et al*., 2003; Walker & Lithgow, 2003; Gerisch & Antebi, 2004; Mak & Ruvkun, 2004). DAF-16 inhibits growth (promotes dauer) and is silenced upon phosphorylation by the insulin effector protein kinase B (PKB or AKT). Like the products of *daf-2* and *age-1*, AKT is active when animals have sufficient resources for growth. In turn, *C. elegans* AKT is regulated by 3-phosphoinositide-dependent kinase (PDK1), a homolog of the mammalian AKT1 kinase, PDK1. Loss of function PDK1 mutations lead to constitutive dauer although this is suppressed by elimination of DAF-16 function. PDK1 transfers signals from AGE-1 to AKT1 and AKT2, resulting in their activation, DAF-16 phosphorylation and promotion of *C. elegans* growth, development and reproduction (Paradis *et al*., 1999) (Fig. 2).

The TGFβ-related signaling pathway influences *C. elegans* dauer entry, although mutations to the pathway fail to extend adult longevity (da Graca *et al*., 2003; Liu T *et al*., 2004; Matyash *et al*., 2004). *Caenorhabditis elegans* gene *daf-7* encodes the TFGβ ligand and it binds receptors DAF-1 and DAF-4, probably in neurons. Direct receptor targets are the Smads DAF-8 and DAF-14 which antagonize the Smad DAF-3 and its cofactor DAF-5 (Fig. 2). In vertebrates, Smad proteins regulate cyclin kinase inhibitor gene transcription, thus influencing cell cycle events. Environmental cues modulating the *C. elegans* TGFβ signaling pathway are similar to those regulating the insulin/IGF mechanism and there is cross talk, with mutational inhibition of the TGFβ pathway decreasing insulin/IGF pathway activity and leading to dauer. Under reduced TGFβ signaling, *daf-2* expression decreases, indicating the insulin/IGF pathway is regulated positively by the TGFβ pathway (Liu T *et al*., 2004).

Although uncertainties persist, the insulin/IGF and TGFβ pathways are thought to merge at DAF-9/DAF-12, the latter a nuclear hormone receptor under control of the steroid hormone synthesized by DAF-9, with TGFβ pathway inactivation coupled to down-regulation of *daf-9* and up-regulation of *daf-12* dauer promoting activity (Figs 2 and 3) (Gerisch & Antebi, 2004; Ludewig *et al*., 2004; Mak & Ruvkun, 2004; Matyash *et al*., 2004). Thus, signaling from

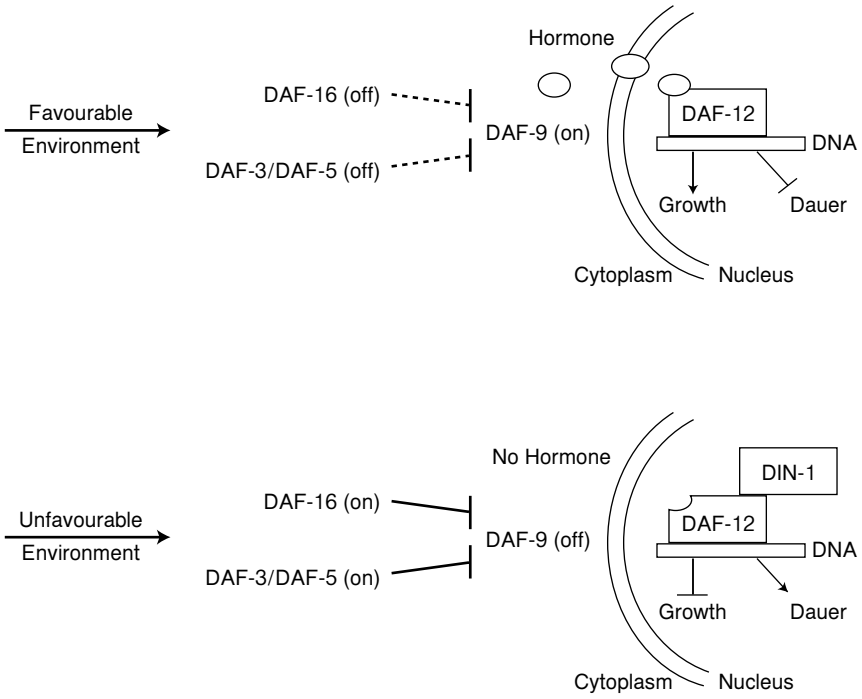

FIG. 3. DAF-9/DAF-12 function during *C. elegans* growth and dauer. Under favorable environmental conditions the insulin/IGF and TGFβ pathways are activated, DAF-16 and DAF-3/DAF-5 are turned off, DAF-9 is active, and reproductive growth occurs (upper figure). In contrast, DAF-12 complexes with the transcription factor corepressor DIN-1S (DAF-12-interacting protein 1 short isoform) as unfavorable surroundings prevail and the steroid hormone product of DAF-9 diminishes, thereby arresting development and promoting dauer. Dashed lines, inactive; solid lines, active. Adapted from Ludewig *et al*. (2004).

TGFβ receptors antagonizes dauer, this potentially accomplished by interaction of the DAF-9 hormone with DAF-12, an idea that remains under investigation (Matyash *et al*., 2004). *daf-9* mutants therefore influence dauer by acting down-stream of both the TGFβ pathway and *daf-16*, and up-stream of *daf-12*. Upon elimination of DAF-9 the hormone that binds DAF-12 is lost leading to growth inhibition and dauer promotion. DAF-12 may support the nuclear import of DAF-16, perhaps through physical interaction of the proteins, and the FOXO transcription factor induces expression of dauer-specific genes (Liu T *et al*. 2004; Matyash *et al*., 2004). In a related possibility, DAF-12 complexes with the transcription factor corepressor DIN-1S (DAF-12-interacting protein 1 short isoform) as unfavorable conditions prevail and the DAF-9 steroid hormone product diminishes, thereby arresting development and supporting dauer (Fig. 3) (Ludewig *et al*., 2004). How transcriptional regulation controls dauer entry in multicellular *C. elegans* larvae is unknown, but the possibility of a hormone signal that traverses the organism is invoked because many cells modified during dauer are not innervated.

DAF-16, a transcriptional regulator of development and longevity

The DAF-16 equivalent in *Drosophila* is FOXO (Neufeld, 2003; Hwangbo *et al*., 2004), a transcription factor under insulin-signaling pathway influence. DAF-16 negatively regulates cell number partly by activation of *d4E-BP*, a gene that encodes an inhibitor of the eukaryotic protein synthesis initiation factor eIF4E (Jünger *et al*., 2003; Moore, 2003). The major function of the insulin signaling pathway in *Caenorhabditis* and *Drosophila* is to regulate growth in response to nutrient supply. As described, *C. elegans* enter dauer when nutrients are limited, while in *Drosophila* growth is modulated by control of cell number, in addition to effects on diapause and aging.

Active *daf-16* is necessary for *C. elegans* life span extension by *daf-2*-insulin/IGF-1 receptor mutations, as is heat shock factor-1 (HSF-1), a transcription factor responsible for directing the heat shock response

(Hsu *et al*., 2003). This parallels the observation that genes up-regulated in dauer and long-lived *daf-2* mutants are often enriched in *daf-16*-binding elements and heat shock elements (HSEs) (Liu T *et al*., 2004a; McElwee *et al*., 2004). Over-expression of *hsf-1* increases longevity whereas decreasing *hsf-1* expression speeds aging and lessens lifespan. *daf-16* is required for *hsf-1* dependent extension of longevity indicating HSF-1 and DAF-16 coordinately promote expression of specific genes, including those for the small heat shock proteins (sHSPs), through binding to gene regulatory elements. *Caenorhabditis elegans* with reduced *daf-2* activity experience a dramatic increase in expression of several sHSP genes, including *hsp16.1*, *hsp16.49*, *hsp-12.6*, *hsp-12.3*, *hsp-20* and *sip-1*, but expression decreases in animals with reduced *daf-16* activity (Hsu *et al*., 2003; McElwee *et al*. 2004). The sHSPs extend life span as shown by RNAi knock back in *C. elegans*, and enhanced longevity may result because sHSPs prevent protein aggregation by acting as molecular chaperones (Hsu *et al*., 2003). Moreover, introduction of extra *hsp-16* genes promotes *C. elegans* stress resistance and longevity, with DAF-16 necessary for maximum HSP-16 synthesis and life span (Walker & Lithgow, 2003).

Transcripts for the molecular chaperones, HSP70 and HSP90, are up-regulated in dauer larvae and in young adults of long-lived *daf* mutants (Cherkasova *et al*., 2000). By using serial analysis of gene expression (SAGE), the *Caenorhabditis* dauer stage was shown to exhibit a complex gene expression pattern with longevity associated transcripts and other novel genes (Jones *et al*., 2001). Among the proteins synthesized abundantly during dauer are *hsp-12.6*, a sHSP that may be induced as development arrests, and a histone variant which potentially enhances chromatin stability and dampens transcription. In related work, over-expression of mitochondrial HSP22 extends *Drosophila* life span while increasing resistance to heat and oxidative stress (Morrow *et al*., 2004a). Concomitantly, eliminating HSP22 reduces longevity by approximately 40% and amplifies stress sensitivity (Morrow *et al*., 2004b).

The *C. elegans* insulin responsive signaling pathway interacts with the TOR (target of rapamycin) protein kinases to regulate larval longevity, metabolism and development (Vellai *et al*., 2003; Jia *et al*., 2004). TOR is a phosphatidylinositol kinase-related kinase inhibited specifically by the macrolide rapamycin. In response to nutrients, TOR influences autophagy (self-digestion), metabolism, translation and ribosome synthesis. The genes encoding S6 ribosome protein kinase and 4EBP1, a translation initiation factor 4E binding protein, are regulated coordinately by insulin signaling and TOR and they control cell growth. TOR interacts with raptor (regulatory associated protein of mTOR) in mammals, relaying nutrient signals to the translation machinery (Hara *et al*., 2002; Kim *et al*., 2002), and in *C. elegans*, *daf-15* encodes a raptor ortholog (Jia *et al*., 2004). The *daf-15* mutants are dauer constitutive with development ceasing at the L3 larval stage, although residual feeding activity remains. The *daf-15* mutant larvae fail to develop into adults, nor do they complete dauer morphogenesis, characteristics shared by animals with mutations to *let-363*, the *C. elegans* TOR gene. Longevity of *daf-15* mutants increases and the expression of DAF-15 depends upon the *daf-2* insulin/IGF pathway via DAF-16. Nutrient and insulin/IGF signaling intersect through these pathways to manage *C. elegans* life span (Jia *et al*., 2004).

Caenorhabditis elegans *quiescence and cell cycle arrest*

Examination of *C. elegans* illuminates aspects of cell division control during development, an important facet of diapause and quiescence, the latter a state of suspended metabolic activity, growth and development (Kipreos, 2004; Saito *et al*., 2004). Six *C. elegans* vulval precursor cells enter quiescence in the first larval stage and withdraw from the cell cycle for two additional stages. Cell cycle mutants perturbing the patterned somatic divisions of vulval cells were analyzed to identify factors maintaining quiescence, demonstrating disruption of cyclin-dependent kinase inhibitor (CKI-1) permits inappropriate division of vulval precursor cells, as do mutations to the *cdc-14* phosphatase gene, which also allow extra divisions of other cells in extended G1. CDC-14 phosphatase hypophosphorylates CKI-1, preventing destruction of this protein required for cell cycle arrest in G1 (Saito *et al*., 2004). Down-regulation of *cdk-4* occurs in dauer larvae and promotes G1 exit, thus reduced expression of the gene contributes to cell division cessation as larvae enter dauer (Liu T *et al*., 2004).

At all stages of development *C. elegans* can enter reversible, anoxia-induced suspended animation, an extreme quiescence where cell growth, division and movement cease, properties exhibited by several other metazoans in the absence of oxygen (Padilla *et al*., 2002; Nystul *et al*., 2003). *Caenorhabditis elegans*

suspended animation occurs in the absence of the hypoxia-induced transcription factor, HIF-1, demonstrating the signaling pathways leading to anoxia- and hypoxia-induced suspended animation are different. During suspended animation the ATP:ADP ratio drops and phosphorylated derivatives of proteins such as histone 3 and certain cell cycle regulated proteins decrease, signifying posttranslational modification of proteins promotes survival during anoxia (Padilla *et al*., 2002). The reversible suspension of functions associated with life in response to anoxia likely entails a discrete sequence of events, including termination of cell growth, such that organisms are able to recover once oxygen is available. As an example, application of RNAi technology reveals the *C. elegans* gene *san1* (suspended animation 1) is active under anoxia and necessary for survival, but is not required during normoxia or hypoxia (Nystul *et al*., 2003). Sequence comparisons show the *san-1* product is related to *Saccharomyces cerevisiae* Mad3p, a spindle checkpoint protein positioned on poleward facing kinetochore surfaces, a common location for these protein types. Additionally, a second spindle checkpoint factor, the product of *mdf-2*, is essential for survival of anoxia-induced suspended animation in *C. elegans* (Nystul *et al*., 2003). Spindle checkpoint components inhibit metaphase to anaphase transition and prevent cells with insufficient energy reserves from entering anaphase. Specific intracellular programs, and not just ATP depletion, are required for survival of anoxia-induced suspended animation.

INSECT DIAPAUSE

Insect diapause and hormones

Day length and temperature regulate diapause in many insects. Additionally, signals such as neuropeptide hormones initiate and terminate diapause, these varying with the species and the developmental stage when the process occurs. Diapause hormone, a twenty-four amino acid peptide product from the diapause hormone and pheromone biosynthesis-activating neuropeptide (Bom-DH-PBAN) gene is under control of a POU transcriptional factor in the female suboesophageal ganglion. The peptide promotes embryonic diapause in the silkmoth *Bombyx mori*, and may influence diapause in *Manduca sexta* (Xu & Denlinger, 2004), but apparently not in other insects (Denlinger, 2002; Zhang *et al*., 2004a). Indeed, in *Helicoverpa armigera*, *H. assulta* and *H. virescens*, diapause hormone-like peptides translated from mRNA encoding five related FXPRLamide peptides terminate diapause, probably through stimulation of the prothoracic glands to produce ecdysteroids which promote continuous development (Xu & Denlinger, 2003; Zhang *et al*., 2004b,c; Zhao *et al*., 2004). The ecdysteroids have a central role in pupal and larval diapause, influencing expression of diapause specific genes by interaction with membrane receptors in *B. mori* and *Sarcophaga crassipalpis*, among other insects (Rinehart *et al*., 2001; Denlinger, 2002; Sonobe & Yamada, 2004), and elevating hemolin production during early diapause in the gypsy moth, *Lymantria dispar* (Lee *et al*., 2000). Decrease in juvenile hormone production in the *corpus allatum* induces diapause in the blow fly, *Protophormia terraenovae* (Shiga *et al*., 2003).

Gene regulation during insect diapause

Many genes are down-regulated and a few up-regulated during insect diapause (Flannagan *et al*., 1998; Daibo *et al*., 2001; Denlinger, 2002; Tanaka *et al*., 2003). The *L. dispar* hemolin gene (Lee *et al*., 2002), and *Scys-B*, which encodes a cysteine proteinase inhibitor or cystatin in *S. crassipalpis* (Goto & Denlinger, 2002), express in early diapause. Others, such as the cytochrome *c* oxidase subunit 1, required for ATP production, are active at pupal diapause termination in the potato hornworm, *Agrius convolvuli* (Uno *et al*., 2004). Some genes operate intermittently during diapause, others continuously (Denlinger, 2001, 2002; Yocum, 2003). The down-regulation of genes encoding proteins that promote cell cycle progression, including proliferating cell nuclear antigen (Flannagan *et al*., 1998), is interesting because insect diapause entails cell cycle arrest at stages varying between species. On the other hand, stress proteins and/or their transcripts, including those for a desiccation stress protein, small heat shock proteins, and HSP70 (Denlinger *et al*., 1992; Flannagan *et al*., 1998; Yocum, 2003), amplify during insect diapause, observations in line with enhanced stress resistance in dormant organisms (Yocum *et al*., 1998; Liang & MacRae, 1999; Rinehart *et al*., 2000; Denlinger, 2002; MacRae, 2003). However, stress proteins examined in *D. triauraria* are not regulated as part of diapause (Goto *et al*., 1998; Goto & Kimura, 2004) and *S. crassipalpis* HSP90 is down-regulated during pupal diapause (Rinehart & Denlinger, 2000), demonstrating differential regulation in comparison to other insects. Molecular chaperones may protect

structural, regulatory and enzymatic proteins required for resumption of development upon diapause termination. Additionally, the expression of genes encoding DNA repair proteins and transcription factors that promote expression of other diapause-specific genes is probable, with the ETS family an example (Suzuki *et al*., 1999).

Genes encoding kinases that either inhibit or promote cell cycle regulatory cyclins and kinases are possibly stimulated during insect diapause (Flannagan *et al*., 1998). Moreover, reversible protein phosphorylation represents a major regulatory mechanism associated with metabolic rate depression in animals undergoing topor, hibernation, anhydrobiosis and diapause. Phosphorylation induces functional modifications to metabolic enzymes, membrane ion channels and receptors, protein synthesis and degradation (Storey & Storey, 2004). Little is known about diapause-related posttranslational modification of proteins, although it likely represents a regulatory process of some importance.

DIAPAUSE IN THE CRUSTACEAN *ARTEMIA FRANCISCANA*

Embryos of the Branchiopod crustacean, *A. franciscana*, live in waters of varying salinity, where they are subject to changes in aeration, uncertain food supplies, temperature fluctuation and drying. To survive these conditions, *Artemia* development occurs either ovoviviparously or oviparously, the former yielding larvae (nauplii), and the latter encysted gastrulae (cysts), which are partly syncytial and contain approximately 4000 nuclei (Fig. 4) (Liang & MacRae, 1999; MacRae, 2003). Both pathways involve five days of post-fertilization development within the female. Released nauplii continue development, eventually becoming adults, whereas cysts are discharged as dormant gastrulae (developmental diapause) and undergo a dramatic reduction in metabolic activity several days later (metabolic diapause). Resumption of cyst growth requires activation, probably by desiccation, followed by hydration and aeration. If circumstances subsequent to desiccation are unfavorable, or if adverse situations are encountered, cysts enter quiescence, a state of suspended animation. Quiescence terminates upon return to supportive environments and there is no need for activation by physical factors as in *Artemia* diapause. Encysted embryos tolerate desiccation, heat, and anoxia exceptionally well, with quiescent embryos surviving fully hydrated at ambient temperature without oxygen for several years (Clegg, 1997; Clegg *et al*., 2000), an ex-

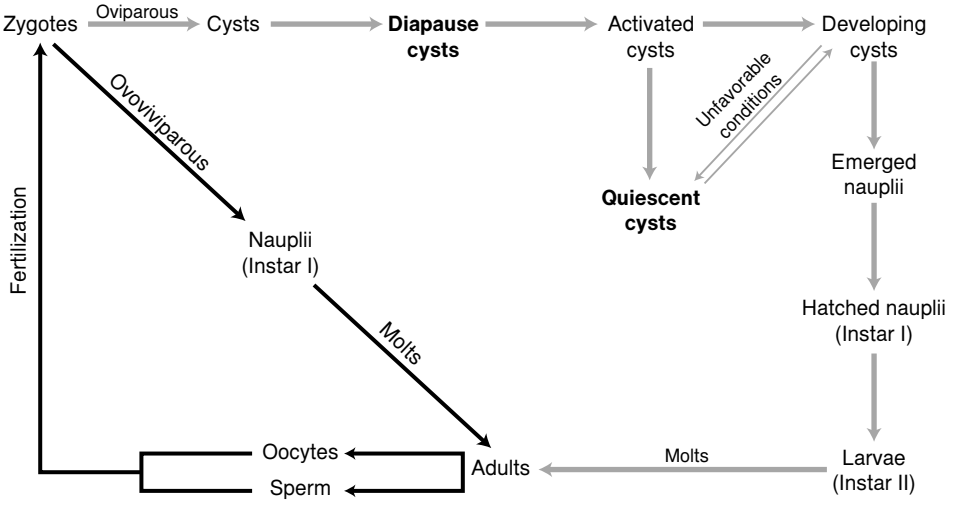

FIG. 4. Development of *A. franciscana*. Fertilized *Artemia* oocytes undergo ovoviviparous development and swimming nauplii (instar 1 larvae) which undergo uninterrupted development into reproductive adults are released from females (left side). Alternatively, oviparous development manifests, embryos encyst as gastrulae (cysts), leave the female, and undergo metabolic arrest upon entering diapause. Cysts survive anoxia, high temperature and desiccation (top), the latter terminating diapause. Activated cysts resume development and produce nauplii in approximately twelve hours. Growth is arrested in unfavorable situations and cysts remain quiescent until stress is relieved, this followed by nauplii emergence (right side). Quiescent cysts survive several years, fully hydrated at ambient temperature, in the absence of oxygen, an extreme level of stress resistance for a metazoan. The figure is adapted from Liang & MacRae (1999).

treme level of resistance for a metazoan. *Artemia* diapause, as in insects, is genetically predetermined, occurring in anticipation of stress, but quiescence represents response to environmental hardship, and from this perspective is superficially similar to *C. elegans* dauer. However, molecular analysis of regulatory events during *Artemia* quiescence, unlike the situation for *C. elegans* dauer, is almost completely lacking.

Artemia cysts are abundantly endowed with the proteins, p26 and artemin (Jackson & Clegg, 1996; Liang *et al*., 1997a,b; Liang & MacRae, 1999; Crack *et al*., 2002; Chen *et al*., 2003; Day *et al*., 2003; MacRae, 2003; Qiu *et al*., 2004; Sun *et al*., 2004; Warner *et al*., 2004). Protein p26, a small heat shock protein, functions as a molecular chaperone *in vitro* and *in vivo* (Liang *et al*., 1997a; Liang & MacRae, 1999; Day *et al*., 2003; Sun *et al*., 2004). This protein is thought to protect against stress and prevent irreversible protein denaturation, thus ensuring a sufficient supply of functional proteins for resumption of embryo development once diapause is broken. Moreover, *Artemia* cysts are predisposed to survive quiescence due to the chaperones and other protective constituents accumulated in anticipation of diapause. As another potential function, p26 binds tubulin and hinders microtubule assembly *in vitro* (Day *et al*., 2003). p26 may therefore disrupt the mitotic apparatus leading to inhibition of cell division and development, characteristics of oviparously developing embryos (MacRae, 2003). This sHSP may also account for the lack of mitosis in developing post-diapause embryos which lasts until emergence and the concomitant major reduction in p26. Additionally, this molecular chaperone populates discrete nuclear locations, possibly protecting important nuclear proteins and modulating functions such as DNA replication or transcription (Liang *et al*., 1997b; Willsie & Clegg, 2002). sHSPs inhibit apoptosis (Liu J-P *et al*., 2004; Mao *et al*., 2004) suggesting a parallel function for p26 as *Artemia* embryos experience stress able to induce programmed cell death.

Artemin is less well characterized than p26 and might protect embryos during diapause and stress. Artemin resembles ferritin, an iron binding protein, in primary and quaternary structure, but molecular modeling indicates artemin fails to interact with metals even though both proteins form oligomers (Chen *et al*., 2003). Artemin associates with RNA at high temperature and may link with RNA *in vivo* (Warner *et al*., 2004). The physiological relevance of these and other observations in the context of embryo diapause remain under investigation.

Cell/molecular studies of oviparous development in *Artemia* are in progress. As a model for transcriptional regulation during oviparous development and diapause, the p26 gene, including upstream regulatory elements, has been sequenced. Among the computer identified regulatory elements several heat shock elements are housed in the 5'-untranslated region and in the initial intron of a gene that is developmentally regulated but not induced by stress (unpublished data). Subtractive hybridization was used to identify up-regulated genes during early oviparous development (unpublished data). Some of the genes encode mammalian tumor suppressor homologues, at least one is a transcription co-factor known to modulate cell proliferation and two are novel small heat shock proteins. Based on these data and the emergence of new methodologies for analysis of gene expression and protein function in *Artemia*, including the application of RNAi (Copf *et al*., 2004), the study of diapause in this unusual organism promises additional interesting findings.

MAMMALIAN EMBRYONIC DIAPAUSE: DELAYED IMPLANTATION AND BLASTOCYST SUSPENDED ANIMATION

The initial physiological and physical contact of mammalian blastocysts with the uterine epithelium, termed implantation, is important for establishing pregnancy and determining pregnancy failure, both significant clinical and agricultural issues. Mammals in several orders experience delayed implantation, known as embryonic diapause. Blastocysts enter suspended animation, representing a neonate survival strategy characterized by slow growth and either limited or complete lack of mitosis and cytokinesis (Renfree & Shaw, 2000; Reese *et al*., 2001; Dey *et al*., 2004; Hamatani *et al*., 2004; Lopes *et al*., 2004). Mammalian embryonic diapause may be facultative, an environmentally induced situation related to continued existence of females and the ability to nourish embryos. In contrast, obligate embryonic diapause takes place every gestation, synchronizing parturition with environmental parameters favoring neonatal survival (Lopes *et al*., 2004). Embryonic diapause is occasioned experimentally by loss of ovarian estrogen through surgical removal of an ovary (ovariectomy) before implantation, followed by pro-

gesterone exposure, and lasts with decreasing embryo survival for about two weeks. The condition occurs during lactation in mice subsequent to postpartum mating, an effect caused by limited ovarian secretion of estrogen, and broken when blastocysts are activated by exposure to the hormone.

Gene expression patterns vary in activated *versus* dormant mouse embryos (Hamatani *et al*., 2004). Microarray analysis demonstrates 229 genes, representing approximately 1% of the total number examined in mice, are differentially regulated, with 149 genes expressed at a higher level in activated embryos and 80 up-regulated during diapause. The cell cycle slows in dormancy and rejuvenates upon activation, events apparently under the control of estrogen-responsive regulatory pathways. Major metabolic enhancement characterizes activated *versus* dormant embryos with pyruvate and glucose driven pathways greater in the former. Dormant embryos, on the other hand, may resemble *C. elegans* dauer larvae where inactivation of the *daf-2* gene product, the insulin/IGF I receptor protein, stops growth and maintains dormancy. Genes involved in Ca^{++} and inositol phosphate signaling, and those encoding karyopherins, proteins involved in nuclear transport, are up-regulated in activated embryos. A role is suggested for these genes during resumption of development, whereas inhibition or down-regulation promotes dormancy. Analysis of gene regulation in dormant and activated mouse embryos affords a baseline for investigation of other species where blastocysts are less readily available. The studies may reveal important characteristics of human fertility and they evoke candidate genes/proteins regulated during diapause in other organisms, including those distantly related to mice.

CONCLUSIONS

The study of diapause brings together many ideas, revealing common biological characteristics between disparate organisms that are not always readily apparent and which typify the interdependence of biological activities from molecular to ecological levels. A striking similarity among cells is the contribution of evolutionarily conserved molecular chaperones to enhanced stress resistance, a property exhibited by organisms in diapause. The hormonal induction of diapause imposed by shifting environmental conditions such as day length, temperature, crowding, and food availability, occurs in various organisms. These signals translate into altered gene expression, tying organisms to their surroundings. The coordinated termination of diapause synchronizes growth and reproduction, allowing effective resource utilization. Diapause affects gene flow within populations because reactivation of organisms that lay dormant for many years introduces individuals from previous time which, depending on the length of the reproductive cycle, may represent many generations. In effect, genomes move through time, without apparent alteration, and mix with contemporary genomes as reproduction occurs. Understanding the many ramifications of diapause will enrich our appreciation of living organisms, and bring applications ranging from treatment of diseases that perturb the cell cycle and protein structure, control of agricultural pests in ways that do not harm the environment, modification of crop plants to enhance stress resistance, and improved aquaculture production, as a partial list. The study of diapause, representing diverse states of developmental and metabolic arrest, clearly has much to offer.

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