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Mechanisms of animal diapause: recent developments from nematodes, crustaceans, insects, and fish

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Hand SC, Denlinger DL, Podrabsky JE, Roy R. Mechanisms of animal diapause: recent developments from nematodes, crustaceans, insects, and fish. *Am J Physiol Regul Integr Comp Physiol* 310: R1193–R1211, 2016. First published April 6, 2016; doi:10.1152/ajpregu.00250.2015.—Life cycle delays are beneficial for opportunistic species encountering suboptimal environments. Many animals display a programmed arrest of development (diapause) at some stage(s) of their development, and the diapause state may or may not be associated with some degree of metabolic depression. In this review, we will evaluate current advancements in our understanding of the mechanisms responsible for the remarkable phenotype, as well as environmental cues that signal entry and termination of the state. The developmental stage at which diapause occurs dictates and constrains the mechanisms governing diapause. Considerable progress has been made in clarifying proximal mechanisms of metabolic arrest and the signaling pathways like insulin/Foxo that control gene expression patterns. Overlapping themes are also seen in mechanisms that control cell cycle arrest. Evidence is emerging for epigenetic contributions to diapause regulation via small RNAs in nematodes, crustaceans, insects, and fish. Knockdown of circadian clock genes in selected insect species supports the importance of clock genes in the photoperiodic response that cues diapause. A large suite of chaperone-like proteins, expressed during diapause, protects biological structures during long periods of energy-limited stasis. More information is needed to paint a complete picture of how environmental cues are coupled to the signal transduction that initiates the complex diapause phenotype, as well as molecular explanations for how the state is terminated. Excellent examples of molecular memory in postdauer animals have been documented in *Caenorhabditis elegans*. It is clear that a single suite of mechanisms does not regulate diapause across all species and developmental stages.

cell cycle; development; diapause; dormancy; metabolism

THE ABILITY OF ANIMALS TO delay development and enter arrested states is widespread across animal groups and provides many advantages for dealing with inconsistent, ephemeral environments. Virtually all major animal phyla have representative species that show some form of dormancy during their life cycles, the primary exception being Echinodermata (78). The focus of this review will be specifically on one type of dormancy termed diapause. Diapause is a programmed arrest of development that is controlled by endogenous physiological factors and may or may not involve a substantial depression of metabolism (41, 42, 44, 46, 70, 72, 78, 79, 109, 115, 148, 162, 172, 182). Depending on the developmental stage, diapause may be hormonally regulated and may occur in response to signaling cues (e.g., photoperiod) that are predictive of forthcoming environmental change. Animals enter diapause under conditions that are conducive to normal growth and development; in other words, the diapause state often precedes the onset of adverse environmental challenges (although in some species, entry can be a direct response to stress). Diapause is generally distinguished from quiescence, which also is a dormant state but is directly imposed on the organism by an unfavorable and acute change in environmental conditions (e.g., anoxia, desiccation).

The biological advantages of diapause are diverse and depend on the species and habitat. Diapausing egg banks in freshwater and marine sediments are beneficial for reestablishing future populations (68, 71, 72, 119). Resting propagules can aid in transport and dissemination of the species to new locations or serve to synchronize favorable environmental conditions with the actively feeding or growing stage of the organism (35). Certainly, entering the state of diapause is a very common mechanism for overwintering; diapause can be entered in response to food limitation or crowding. Ultimately survival of deleterious environmental conditions is improved markedly by diapause.

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As will be apparent from this review, the diverse experimental models offer unique advantages (or in some cases disadvantages) for investigating various aspects of diapause; typically, all facets cannot be tackled conveniently with a single diapausing form. For example, the power of molecular genetics and mutant strains offered with *Caenorhabditis elegans* has been useful in providing a clear picture of signaling pathways during dauer formation. Mechanistic investigations of metabolic arrest and mitochondrial bioenergetics during diapause can be addressed particularly well with embryos of the brine shrimp, which display the most profound degree of metabolic depression ever measured during diapause. Differences in obligate vs. facultative diapause, as well as insights into diapause across multiple developmental stages, have been highlighted from work on insect species. The diapause phenotype in annual killifish allows resolution of divergent developmental trajectories that provide novel insights into vertebrate developmental plasticity. Such advantages of alternative animal models in research bring to mind the quote of the Danish comparative physiologist August Krogh (105): "For such a large number of problems there will be some animal of choice or a few such animals on which it can be most conveniently studied."

Across all model species evaluated here, one emerging theme in common is the use of small RNAs for diapause regulation. Others include chromatin/histone modifications (brine shrimp embryos, nematodes), reliance on insulin/FoxO signaling (nematodes, insects), and cell cycle arrest by cyclin-dependent kinases (some insect species, embryos of the brine shrimp). Chaperone-like activity appears very important for stabilizing macromolecules during diapause when synthesis of new maromolecules often can be downregulated for extended periods. This review summarizes advancements in understanding diapause that have emerged from experiments on nematodes, crustaceans, insects, and fish. These groups contain the best physiologically characterized examples of animal diapause currently available.

Nematodes

Ecological role for nematode diapause. The Nematoda possess various diapause-like states that greatly facilitate their ability to occupy niches of varying extremes. Perhaps the best characterized of these stages is the dauer larva, which is an obligate phase in the life cycle of many parasitic nematodes as they transit through harsh environments. Regardless of the species, execution of dauer development is associated with numerous morphological and physiological changes, many of which improve the biological barriers between the parasite and its host. This allows the larva to survive in environmental conditions where other organisms could never thrive, allowing them to inhabit niches that range from equatorial rainforests to Antarctica.

This developmental adaptation is not exclusive to the parasitic nematode, and it is found in at least some free-living nematode species, including *C. elegans*. Many of the characteristics involved in the execution, maintenance, and recovery from the dauer stage have been genetically characterized in this organism and have provided more than two decades of insight regarding organismal adaptation to environmental duress (161, 162). In the sections that follow, the majority of functional studies have been performed with *C. elegans*.

Signaling pathways for the dauer stage. Animals form dauer larvae during periods of suboptimal growth conditions following an assessment of resources and population density (Fig. 1). Three independent genetic pathways that are regulated by cGMP, TGF-β, and insulin-like signals act in parallel to determine whether a larva will undergo continuous development or exit this pathway and execute the dauer stage. Neural inputs are paramount to this decision, as the ligands responsible for signaling this decision are all expressed in head neurons, ultimately ensuring that environmental sensing is linked to the adaptive changes that occur at the organismal level (33, 99, 159).

These three signaling pathways converge on a vitamin D receptor-like nuclear hormone receptor called DAF-12 (Dauer Formation abnormal) (7). Unliganded DAF-12 triggers the developmental changes associated with dauer development through its transcriptional modification of a suite of genes required for the associated morphological and behavioral changes that occur in the dauer stage (57). In addition, DAF-12 must also modify genes that impinge on the metabolic program to enable the dauer larva to survive long durations (4–6 mo) without eating (191).

Histone modifications, small RNAs, MicroRNAs. Transit through the dauer stage does not have dramatic consequence on postdauer morphology nor does it adversely affect fitness, although certain features distinguish animals that experienced the dauer stage from those that developed otherwise. Examination of histone modifications associated with various states of gene expression indicate that the genomic distribution of

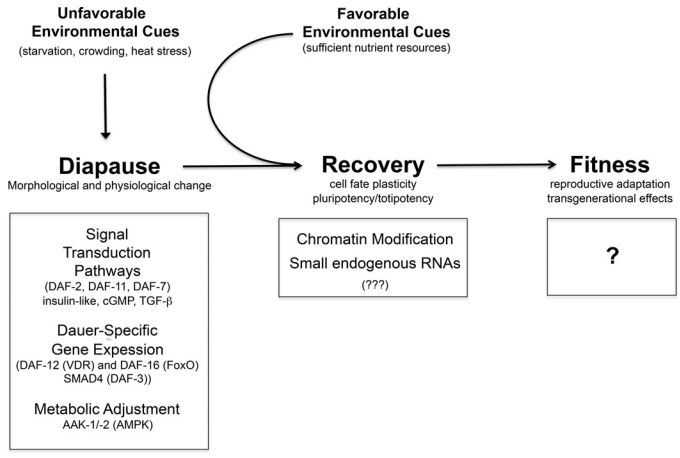

Fig. 1. A molecular summary of dauer and its consequences in *Caenorhabditis elegans*. The diapause-like dauer stage is regulated by signal transduction pathways that control the decision between continuous or dauer development. Key effectors of each of these pathways are indicated: DAF-2, an insulin-like receptor; DAF-11,-a guanylyl cyclase transmembrane receptor required for chemosensory signaling and the cGMP regulatory branch of dauer formation; and DAF-7, a TGF-β-like molecule. Execution of dauer development results in a change in gene expression that is mediated by transcription factors that adjust gene expression to mediate developmental and physiological modification. Upon recovery, the dauer genome is altered compared with nondauer larvae, which is reflected in the modified distribution of chromatin marks and the small RNA population. These changes likely change the genomic ground state, allowing for the correction of specific mutant phenotypes. Precisely how these effects on gene expression and metabolism impinge on brood size and lifespan remain to be elucidated.

these marks is dramatically different compared with the same histone/chromatin signatures in animals that did not transit through dauer development (75). Curiously, levels of both acetylated and methylated H3K4, histone modifications that are generally associated with euchromatin, are significantly lower in postdauer animals and correlate with gene expression data, while levels of H3K9 methylation and H3K36 trimethylation, marks that often correlate with heterochromatin, remain relatively unchanged (refer to *Crustaceans*, *Arrest of the cell cycle, transcription, and protein synthesis*). Reproducible changes in differentially expressed transcripts (clustered by several gene ontogeny terms) are observed, potentially conferring some adaptive advantage. Consistent with this, postdauer adult life span is extended, while animals that transit through dauer also produce more progeny (75).

In addition to these altered chromatin marks, the population of small RNAs (endo siRNAs) present in the postdauer recovered adult animals also seems to be distinct from the small RNA repertoire identified in animals that did not transit through dauer (76). It is currently thought that these small RNAs generally act as beacons for appropriate chromatin writers to mark genomic regions for expression or silencing. Compromise of the key effectors of these RNA-mediated effects alter the epigenetic landscape by changing the genomic distribution of histone modifications associated with gene expression.

It is presently unclear whether these dauer-specific modifications confer changes in brood size and whether this provides some long-term adaptive fitness advantage to postdauer reproductive adults or to subsequent generations. Nevertheless, these small RNAs, along with changes in the distribution of histone modifications that occur postdauer, provide a molecular genetic account of the animal's experience in the dauer state.

The molecular memory that accompanies postdauer animals reflects changes in the ground state of the expressed genome. There are two well-documented situations where this phenomenon has surprising functional consequences and may indeed be linked to the above-mentioned dauer-specific molecular modifications.

The *C. elegans* heterochronic mutants, best known for their microRNA-mediated genetic regulatory hierarchy, exhibit defects in the temporal sequencing of stage-specific events, such as cell division and fate specification (163). These developmental abnormalities often result in visible defects in the hypodermal stem cell lineage and also during vulval formation in the growing animal. The vulval phenotypes are both penetrant and heritable, and yet if the mutant animals execute dauer development, they are significantly suppressed following recovery. The changes in vulva development are dependent on an inherent developmental plasticity that allows the vulval cells to become reprogrammed and, therefore, competent to developmental cues following dauer recovery (54). Maintenance of this capacity is dependent on the *C. elegans* FoxO transcription factor DAF-16, a factor involved in maintaining pluripotency in higher organisms (96, 203) (refer to *Insects*, *How to generate the diverse phenotype of diapause*).

Independent of these studies, investigators involved in characterization of the *C. elegans* LSD1 demethylase orthologue *spr-5* showed that genetic compromise of demethylation results in abnormal levels of methylation at both H3K4 and H3K9 that caused a progressive transgenerational extinction of the germline over multiple generations (66, 97). Surprisingly, this progressive sterility could be reset if the animals passed through the dauer stage (97).

Both of these dauer-suppressible phenotypes suggest that as the animal transits through dauer, the various pathways that are active during this state affect gene activity in an unprecedented manner; by altering the global genomic readout of the animal. In specific contexts, passage through dauer is sufficient to reestablish correct physiological/developmental homeostasis, potentially through the same dauer-specific changes that alter the chromatin marks and the small RNA repertoire, although this remains to be demonstrated. The mechanisms associated with this effect have not been fully characterized, but they must act downstream of all three genetic pathways that control dauer entry, as the suppression has not been shown to be pathway-specific.

AMPK as a downstream effector. Just as DAF-12 is pivotal for dauer entry, AMPK is likely to be the critical downstream effector for each of these dauer formation pathways to ensure that the appropriate allocation of energy resources occurs following the commitment to nonfeeding (137). Through its regulatory phosphorylation of key protein substrates that are involved in both anabolic and catabolic processes, in addition to its effects on nonmetabolic targets, AMPK enhances the levels of available cellular energy to maintain the appropriate function of essential cellular processes (87). In most organisms AMPK signaling is essential (24, 108, 187); however, in *C. elegans* null mutations that remove all AMPK signaling are viable. This provides a unique means of evaluating the role of this critical protein kinase in various physiological and developmental contexts, including dauer development.

A dominant negative variant of the AMPK catalytic subunit *aak-2* that disrupts most AMPK signaling in mutants was isolated in a genetic screen designed to identify regulators of germline stem cell quiescence during the dauer diapause (137). These animals are viable, form dauer larvae, and are fertile if not subjected to stress during growth and development. However, during the dauer stage, the germ cell lineage undergoes significant hyperplasia, almost quadrupling its germ cell numbers during diapause when all cell divisions should be arrested. Some AMPK mutant animals show defects in dauer entry and maintenance (8), while insulin-signaling defective dauer larvae that lack AMPK are incapable of enduring long periods in this stage without feeding and expire after ~10 days (138). This premature dauer-dependent lethality is due to the untimely exhaustion of triglyceride stores that act as a long-term energy source to fuel cellular reactions during the extended period without feeding. During dauer entry AMPK phosphorylates a rate-limiting triglyceride lipase called ATGL-1 to block it from rapidly breaking down the accumulating triglyceride so it can conserve energy resource for long durations. In the absence of AMPK, the animals exhaust their fuel reserves prematurely, compromising energy-dependent organ systems leading to expiration (138).

AMPK, therefore, plays an important, if not essential, role during the dauer stage, and while DAF-12 readjusts gene expression to accommodate the dauer-specific developmental changes, AMPK will alternatively drive the appropriate physiological and metabolic modifications necessary to survive the

stress of dauer (refer to *Crustaceans*, *A role for pH_i or AMPK?*).

Other resting stages. Although the dauer stage is the most thoroughly studied diapause stage in *C. elegans*, several other periods of postembryonic developmental arrest have been described as additional diapause-like states. *C. elegans* transits through four molts before achieving reproductive maturity. Progression beyond each of these developmental interruptions is contingent on sensing or access to adequate environmental resources to support the stage-specific processes that will be engaged once committed to development through the larval stage. Lack of food following the L2, L3, or the L4 molt will result in extended periods of developmental arrest that are dependent upon stage-specific gene activities (168).

If late fourth larval stage animals are starved, they will molt to the adult stage but preempt the formation of embryos and execute an adult reproductive diapause (6). If starvation is prolonged, the germ line undergoes a dramatic shrinkage, while the numbers of germ cell nuclei are also reduced due to caspase-dependent programmed cell death. Despite these major changes in morphology, the germ line retains its totipotent capacity, and when the animal begins to feed again, the germ line is quickly restored to its wild-type stature. Fitness is, however, affected as brood size is reduced significantly as a function of the duration of starvation (6).

As animals emerge from the embryo, the resulting hatchlings, or by convention the L1 larvae, are capable of surviving for up to 2–3 wk without food. During this period the animals are motile but the postembryonic developmental program is never executed without satisfying a nutrient/energy contingency. Transcriptional levels are low and no cell divisions occur until the animals begin to eat, which sets in motion the first features of the postembryonic developmental program (13, 59). This period of cell cycle/developmental arrest is referred to as the L1 diapause and is likely a critical adaptive feature in the wild, where animals are more likely to be born into nutrient-depleted rather than nutrient-abundant environments.

Analysis of the transcriptional state of the RNA polymerase II complex during this period indicates that the transcriptional complex appears bound near transcriptional start sites similar to developmentally regulated genes in *Drosophila* (12, 106). The expression of genes involved in various stress or starvation responses tend to be "paused" in a postinitiated/preelongation state, whereas genes that are associated with growth and development are in a postrecruitment "docked" state (121).

Although this diapause-like state is seemingly very different than formation of the dauer, DAF-16/FoxO also plays a critical role during this arrest. DAF-16/FoxO activates the expression of *cki-1*, a p27-like cyclin-dependent kinase inhibitor protein that ensures cell cycle quiescence during this period (11, 13). In addition to DAF-16/FoxO, the microRNA miR-71 also regulates *cki-1* during the L1 diapause, but in contrast to DAF-16/FoxO, it blocks *cki-1* function to activate cell cycle progress through a DAF-16/FoxO-independent mechanism (203). The role of miR-71 extends beyond simple cell cycle control: it is required for survival of the L1 diapause. Loss of function mutations in miR-71 demonstrate postembryonic defects in developmental timing and vulval patterning that are exacerbated with extended durations in the L1 diapause (203) (refer to *Crustaceans*, *Arrest of the cell cycle, transcription, and protein synthesis*; *Fish*, *Maternal versus embryonic control of entrance into diapause II;* for insects, see *Perspectives and Significance*).

Survival of the L1 diapause is dramatically reduced in animals that lack AMPK signaling, in line with its role in adjusting energy reserves to address the stress associated with starvation. The mutant larvae die prematurely, and they all exhibit supernumerary primordial germ cell divisions, suggesting that as in the dauer stage, AMPK ensures that germline stem cells do not divide during periods when inadequate resources are available to fuel critical cellular processes (13, 59, 60). The fitness consequences of these extra divisions are not clear, but resolving how the targets of this important protein kinase maintain cell cycle arrest in the context of the germ line may provide valuable insight in our understanding of how stem cells maintain their arrest in diverse physiological contexts.

Although these diapause states may be specialized for the *C. elegans* life cycle, it is quite plausible that similar regulatory contingencies are conserved among other species, even if the developmental switch to a diapause stage per se may not be. The dauer stage is an obligate life cycle phase in many parasitic nematodes, while it seems to have evolved environmental triggers in free-living species. The L1 diapause seems to be mirrored by a similar nutrient/energy-dependent arrest that occurs in *Drosophila* (19). This developmental "checkpoint" maintains arrest in the emergent larva until conditions are adequate to support the rapid growth phases associated with the early stages of larval development in the fly. Whether this is conserved beyond these two organisms remains to be demonstrated.

The suppression of reproduction typical of adult reproductive diapause of *C. elegans* may be a more common phenomenon that is shared across species from *C. elegans* to humans, probably as a means of protecting germ lineages from potential defects associated with ongoing cell division during periods of energy or nutrient stress. Characterization of how these important developmental decisions are triggered and how the gene products involved impinge on the appropriate physiological effectors in each of these situations will undoubtedly contribute to our understanding of how cells transduce environmental information to effect cellular and organismal processes from stem cell biology to metabolic regulation: both of which may have multiple future applications in both biotechnology and medicine.

Crustaceans

This section begins by emphasizing the considerable diversity of diapause states among crustacean species and the various environmental conditions under which diapause is induced. While mechanistic inferences can be gleaned from these wide-ranging studies, most molecular, biochemical, and physiological data addressing diapause mechanisms for crustaceans have utilized developmental stages of the brine shrimp *Artemia franciscana*. Consequently, the biology of this organism is concisely reviewed, and the enormous biomass of diapausing animals seen in nature is underscored. The extreme bioenergetic transition observed in the embryos of this species is evaluated and the implications for apoptosis are considered. Mechanisms for the arrest of the cell cycle and transcription and protein synthesis are reviewed, as is the protection and

longevity of biological macromolecules during diapause. Finally, effectors and cues promoting diapuase are briefly considered. Open questions still remaining are interwoven within these topics.

Diversity of diapause among crustaceans. As pointed out by Hairston and Kearns (72), it has been known for almost 140 years that freshwater crustaceans produce diapausing eggs. Indeed, most species of limnic zooplankton produce a resting stage at some point during their life cycle, a process that has been well studied among copepods and branchiopods (58, 69, 71–73, 78, 156). Prolonged egg diapause is observed in 62% of crustacean species living in inland water habitats (72). Freshwater calanoid copepods alternate between production of subitaneous (immediately hatching) and diapausing eggs. Subitaneous eggs are carried by females until they hatch in a few days, whereas diapausing eggs are held for 2 or 3 days and then released to the bottom of the pond where they remain developmentally arrested until hatching the following year (74). In the majority of cases, production of diapausing eggs is environmentally cued by short photoperiod and declining temperature, with crowding and food availability occasionally exerting some influence (69). In contrast, eggs of freshwater cyclopoid copepods are all subitaneous (34), and the arrest occurs later in the life cycle at the copepodite stages (51). Among freshwater cladocerans (water fleas), parthenogenetic eggs can be produced for several generations that hatch exclusively into females. Males (parthenogenetically derived) are usually needed for the fertilization and production of diapausing eggs (69). In genera like *Daphnia*, diapausing eggs are protected by an envelope termed an ephippium that is derived from the walls of the adult carapace. As with freshwater copepods, the most commonly reported cues that induce diapause in cladocerans are photoperiod, temperature, and crowding (69). Finally, marine calanoid copepods are also noted for their abilities to produce diapausing eggs (53, 119, 120, 167); as summarized by Engel and Hirche (53), resting eggs have been reported for 49 species of marine and estuarine calanoid copepods. Marine copepods can exhibit diapause at various stages of the life cycle depending on the species (68, 88, 117). Temperature, photoperiod and deoxygenated water have all been implicated in the maintenance and/or release from diapause (117, 118).

For crustaceans a majority of studies on physiological and molecular mechanisms of metabolic and developmental arrest have used embryos of the brine shrimp *Artemia* (Branchiopoda: Anostraca), due largely to the commercial availability of encysted, anhydrobiotic embryos (78). More importantly in the context of diapause, enormous quantities of hydrated, diapausing embryos can be readily collected from the surface of hypersaline waters like the Great Salt Lake (Utah) (Fig. 2), the fourth or fifth largest terminal saline basin in the world (14). Diapausing cysts must be collected in the hydrated state, because drying is one cue that serves to break the diapause state (see below). Annual biological production of embryos from the lake has been estimated at ~4,500 metric tons of cyst dry mass, of which the commercially harvested fraction can range from 21% to more than 40% (199). During an above-average year in 1996, 2,500 metric tons of dried cysts were harvested (refer to Ref. 199). These dried cysts serve as a ready source of free-swimming nauplius larvae for the fish aquaculture industry and tropical fish hobbyists.

In autumn ovigerous females of the brine shrimp *A. franciscana* shift from ovoviviparous reproduction (eggs hatched internally; live bearing of young), where free-swimming nauplius larvae are released directly into the water column, to oviparous reproduction (eggs develop and hatch outside maternal body), where encysted embryos at the late gastrula stage (15) are released in diapause (29, 32, 46, 78, 107). For the Great Salt Lake (Utah) population, it is clear that photoperiod is the overriding cue that shifts the mode of reproduction to oviparity and diapause (136). For a detailed diagram of the *A. franciscana* life cycle, please see Ref. 148. The diapausing embryo serves as an overwintering stage during which development ceases and metabolism is severely depressed (30, 142). Some of the diapausing embryos are blown toward the shore-

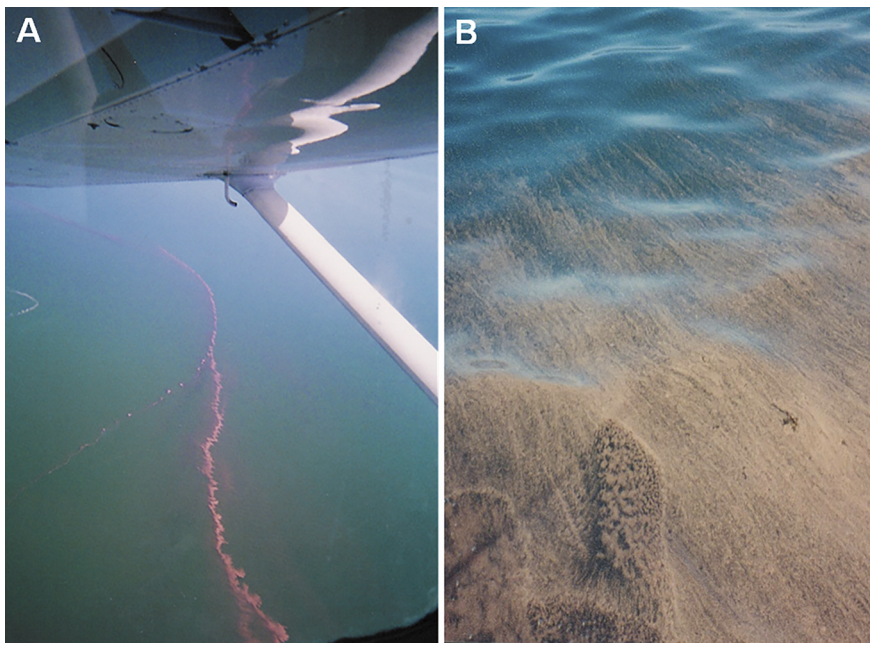

Fig. 2. *A*: aerial view of diapausing embryos of *Artemia franciscana* floating in wind-blown "streaks" on the Great Salt Lake, Utah, in autumn. The streaks of embryos typically are kilometers long. *B*: kilogram quantities of diapausing embryos are easily collected from these streaks on the lake surface.

line in wind rows (streaks; Fig. 2*A*) where they accumulate on the bank and desiccate, while others remain in the lake and overwinter in the hydrated state (c.f., 142).

Bioenergetics. In this and subsequent sections, the majority of functional studies has been restricted to *Artemia franciscana* thus far; whether findings are broadly applicable to other crustaceans is not clear at present. Details of the proximal mechanisms by which metabolism is downregulated during diapause in *A. franciscana* have been reviewed recently (148). Briefly, one major contributor to metabolic arrest is the blocked delivery of carbon substrates to the mitochondrion. During preemergence development of *A. franciscana*, the primary fuel for the embryos is the sugar trehalose (23, 25, 49, 52, 133). Inhibition of several enzymatic steps in the catabolism of trehalose and its delivery to the TCA cycle occurs during the metabolic arrest that is spread over a period of days (142). These enzymes are trehalase, hexokinase, pyruvate kinase, and pyruvate dehydrogenase (PDH), and the restricted metabolic steps were identified by comparing product to substrate ratios that were determined by quantitative measurement of pathway intermediates in diapausing versus post-diapause embryos (Fig. 3). The molecular explanation for inhibition at each step has not been determined, but phosphoryation of PDH occurs with a time course virtually identical to the severe arrest of embryo respiration during diapause entry. This phosphorylation event is known to be associated with strong inhibition of PDH from many sources (see Ref. 142 for details). During the first few days after release of diapausing embryos from ovigerous females, oxygen consumption by embryos plummets, eventually reaching values $\leq$1% of the active state after 2–3 wk (30, 142). This metabolic arrest is the most extreme ever reported for a diapausing animal measured under normoxia, euthermia, and full hydration. Indeed, Clegg and Jackson (31) argue persuasively that metabolism eventually may be brought to a reversible standstill during diapause, based on unchanged carbohydrate reserves during 17 mo of diapause. By comparison, a meta-analysis for 15 marine copepod species has shown that metabolism in diapausing copepodid stages is reduced to about one-fourth of that measured for actively growing copepods (116).

Proton conductance across the inner membrane of the mitochondrion does not change during diapause versus the active state (142). Consequently, it would be anticipated that both the ΔpH and $\Delta\Psi$ dissipate (Fig. 4). This loss of proton-motive force during diapause has major implictions for ATP metabolism (142, 148), because it is possible that the F_1F_o ATP synthase could reverse under such conditions and become an ATPase. What makes this possibility particularly troubling is that the adenine nucleotide translocator (ANT) could also reverse, thereby importing ATP into the matrix from the cytoplasm (Fig. 4). Directionality of the ANT normally depends on the presence of $\Delta\Psi$, due to the difference in charge between the two molecules exchanged (ATP^{4-}, ADP^{3-}). If the $\Delta\Psi$ is compromised, directionality then only depends on the respective concentration gradients. Thus, ATP entering from the cytoplasm could be continuously cleaved by the ATPase. One possible mechanism that might serve to prevent the reversal of the ATP synthase would be binding of IF_1 protein

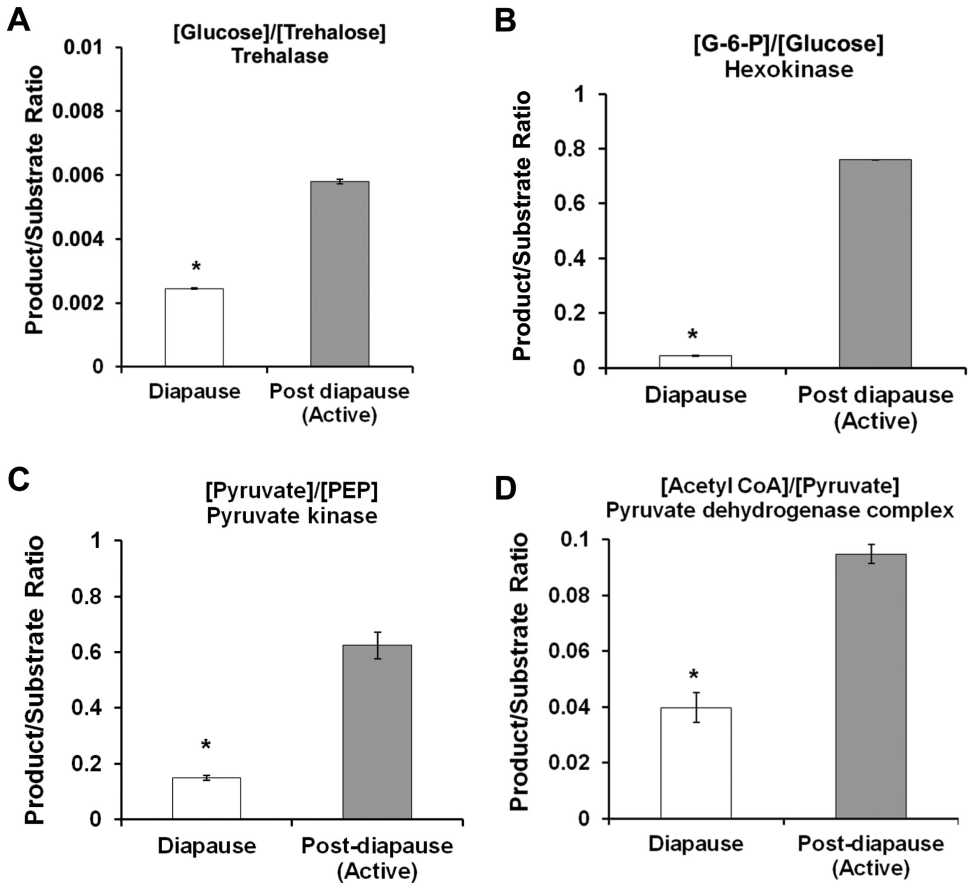

Fig. 3. Product-to-substrate ratios for diapausing embryos (open bars) of *Artemia franciscana* compared with postdiapause embryos (shaded bars). The significantly lower values seen in diapause indicate inhibition at the trehalase (*A*), hexokinase (*B*), pyruvate kinase (*C*), and pyruvate dehydrogenase (*D*) reactions in the pathway for trehalose catabolism. Values are expressed as means $\pm$ SE for $n = 4$ samples in *A*–*C* and $n = 6$ for *D*. *Statistical significance with $P < 0.0001$. [Modified from Patil Y, Marden B, Brand MD, SC. "Metabolic downregulation and inhibition of carbohydrate catabolism." *Physiol Biochem Zool* 86: 111–113, 2013, published by the University of Chicago; Ref. 142].

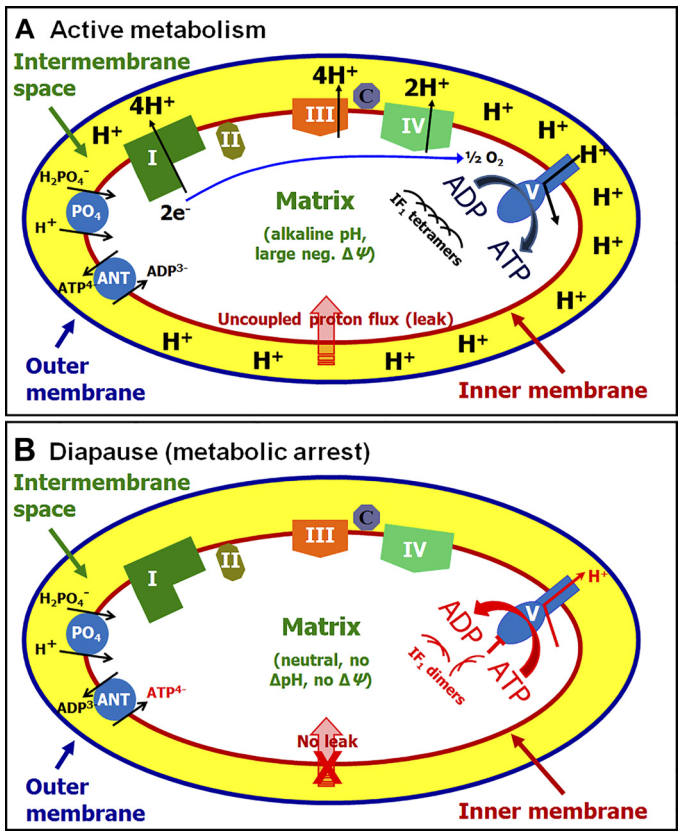

Fig. 4. Aspects of mitochondrial energetics during active metabolism (*A*) vs. the diapause state (*B*), as predicted for embryos of *Artemia franciscana*. *A*: the TCA cycle and electron transport system (ETS) are active with high oxygen consumption measurable. Under this condition, a large ΔpH and ΔΨ would be predicted with an alkaline pH present in the matrix. The F_1F_o ATP synthase (ComplexV) operates in the direction of ATP production, and the adenine nucleotide translocator (ANT) and phosphate carrier would run in their typical directions. Alkaline conditions in the matrix could potentially foster the tetrameric (inactive) state of the inhibitor protein IF_1. *B*: during diapause, carbon delivery to the mitochondrion is arrested and the TCA cycle, ETS, and respiration are depressed, which would be predicted to result in loss of membrane potential and proton gradient, because proton conductance is known not to change during diapause compared with the active state in mitochondria from *A. francicana*. Consequently, the F_1F_o ATP synthase and ANT could potentially reverse, leading to consumption of cellular ATP stores. Proton leak would promptly cease due to loss of ΔpH. However, under the more acidic conditions in the matrix, one might hypothesize that the IF_1 protein could depolymerize to active dimers and bind to the F_1F_o ATP synthase, thereby preventing reversal of Complex V.

that is known to block ATPase activity under conditions when the matrix pH falls (refer to 10, 56, 63). Under such conditions, the IF_1 from mammalian sources can depolymerize from inactive tetramers into active dimers. Whether the IF_1 protein is involved in potentially slowing the drop in ATP during diapause, and if so how, remain open questions.

Compared with postdiapause embryos, ATP drops significantly during diapause in *A. franciscana* embryos but substantial quantities of ATP remain (142). The ATP:ADP ratio in fully hydrated, diapausing embryos is 1.31 ± 0.04 (means ± SE) vs. 7.3 ± 0.28 for postdiapause embryos. In another report of adenylate values purportedly for diapausing embryos of *A. franciscana* (207), the cysts were allowed to dry for 2 wk at 25°C prior to analysis; this treatment routinely breaks the diapause state and disrupts adenylate status. Not surprisingly, the reported ATP:ADP ratio was extremely low. Likewise the ATP:ADP ratios for postdiapause embryos were low (207) compared with other published values (e.g., 3, 142), likely due to the lack of tissue freeze-clamping in liquid nitrogen during extraction. Declining ATP is a classic signal that often initiates intrinsic apoptosis as documented for a number of mammalian cell lines (refer to Refs. 65 and 81). The intrinsic pathway, which involves mitochondrial signaling, is a response to moderate perturbation of intracellular homeostasis by various cellular stresses. Some of the mechanisms thought to contribute to avoidance of cell death during this energy-limited state have been reviewed previously for *A. franciscana* embryos (81, 126). Of note here is that mitochondria of these embryos are refractory to signals that typically open the mitochondrial permeability transition pore (MPTP), leading to release of proapoptotic factors into the cytoplasm. The lack of a MPTP and the lack of cytochrome-*c* effects on caspase activation (127) are likely significanct for avoiding unwanted cell death during diapause in these embryos. It should be noted that nothing is known presently about the existence in *A. franciscana* embryos of the *Bax*/*Bak* mechanism for mitochondrial outer membrane permeabilization, which is a second mechanism for release of proapoptotic factors and initiating intrinsic apoptosis (104). These observations certainly do not preclude apoptosis in these embryos, because the extrinsic pathway to apoptosis, activated through ligation of death receptors located in the plasma membrane, frequently serves as a mechanism to eliminate cells during development, differentiation, and tissue remodeling, as tissues are removed and replaced (81). Under certain experimental conditions with selective protein knockdown, terminal deoxynucleotidyl transferase dUTP nick end labeling assays have shown apoptosis to occur in developing embryos of *A. franciscana* (110, 202, 204). It is, nevertheless, interesting that during energy-limited states like diapause and anoxia, these embryos survive for months to years without apoptosis.

Arrest of the cell cycle, transcription, and protein synthesis. Once environmental cues have signaled expression of the oviparous developmental pathway (29, 32, 46, 107), cell division ceases in diapausing embryos (134, 135, 141) at the late grastrula stage (15) at about 4,000 cells (134, 140). Even after diapause is terminated and development is reinitiated, the cell cycle remains arrested throughout preemergence development (PED), i.e., until the embryo begins to emerge from the cyst to eventually form the free-swimming nauplius. Thus during PED, there is cell differentiation without cell division or DNA synthesis (for review, see Ref. 29). The precise point in the cell cycle at which arrest occurs is still a matter of some uncertainty, but recent information suggests G_2/M phase during PED (40).

Global mechanisms that integrate the environmental cues with cell cycle and metabolic arrest during diapause in *Artemia* embryos are lacking. Some progress has been made regarding the proximal signaling pathways that may govern induction and maintenance of mitotic arrest during diapause and PED. The phosphorylation state of Polo-like kinase 1 (Plk1) has been reported to decline during diapause in *Artemia parthenogenetica*, rendering the enzyme inactive, but its phosphorylation increases again when mitosis returns in nauplius larvae (110). Knockdown of Plk1 in developing embryos disrupts mitosis and leads to formation of "pseudo-diapause" cysts. The impact

of Plk1 during active mitosis was concluded to be inhibition of p90 ribosomal S6 kinase 1 (RSK1; i.e., Ar-RSK1) via blockage of its upstream activation pathway, MEK-ERK (mitogen/extracellular signal-regulated kinase; extracellular signal-regulated kinase). This linkage between Ar-RSK1 and the MEK-ERK signaling pathway required reevaluation when it was discovered that Ar-RSK1 does not possess a docking site for ERK (48). Rather, a second RSK protein was found in *A. franciscana* embryos (Ar-RSK2) that does possess the docking site. The current model is that Ar-RSK2 is downregulated during diapause, which promotes mitotic arrest. In actively developing embryos, knockdown of Ar-RSK2 led to decreased levels of cyclin D3 and phosphorylated histone H3, and the production of pseudo-diapause cysts. Phosphorylation of Ar-RSK1 is now thought to block meiosis in *A. franciscana* oocytes (48). Most recently, expression patterns of microRNAs have been reported for *A. parthenogenetica* (205). Evidence indicates that miR-100 and miR-34 are involved in cell cycle arrest during diapause. Specifically, miR-100 and miR-34 target Plk1, which leads to activation of the MEK-ERK-ArRSK2 pathway and cyclin K, respectively. RNA polymerase II activity is suppressed as a result (refer to sections on *Nematodes*, *Other resting stages*; *Fish*, *Maternal versus embryonic control of entrance into diapause II;* for insects, see *Perspectives and Significance*).

Histone modification during diapause has not been thoroughly studied to this point in brine shrimp. Changes in histone acetylation have been reported during entry and exit from diapause in embryos of *A. parthenogenetica* (206). Specifically, acetylation of lysine 56 in histone H3 (H3K56ac) increases during diapause entry and declines during diapause termination. H3K56ac remains at basal levels throughout subsequent embryo development. From these data alone, it is not possible to definitively conclude that this acetylation event promotes cell cycle arrest during diapause, because, as previously discussed, cell cycle arrest continues after diapause breakage through the end of PED; yet, H3K56ac returns to basal levels after diapause termination. Treatment of developing embryos during PED with nicotinamide (a deacetylase inhibitor) increases H3K56ac and promotes a temporary developmental arrest immediately before emergence; at emergence, cell division is required to proceed onward to larval stages. More work is needed to clarify the molecular nature of the respective cell cycle arrests seen in diapause versus post-diapause embryos to resolve the role of histone acetylation. Finally, changes in histone methylation apparently occur during development of *Artemia sinica* based on the recent characterization of a protein arginine N-methyltransferase 1; any specific role in diapause has yet to be evaluated (93) (refer to *Nematodes*, *Histone Modifications, Small RNAs, MicroRNAs*).

The best experimental evidence for arrest of nucleic acid synthesis during diapause in *A. franciscana* embryos comes from quantifying the incorporation of radiolabeled precursors into the nucleic acid fraction of diapause versus activated embryos (30). Incorporation into nucleic acid was strongly depressed in diapause embryos, which indicates cessation of DNA and RNA synthesis (for review of earlier literature, see Ref. 175). It is important to note that severe depression of nucleic acid synthesis was not seen in diapausing embryos that were recently released from the females (i.e., after 0–3 days), but only for embryos 2 wk postrelease. This pattern mirrors the asymptotic depression of metabolic rate described earlier and is likely a result of time-dependent arrest of RNA synthesis. A cyclin K-dependent mechanism that may decrease transcription during diapause in *A. parthenogenetica* has been reported (204). Briefly, cyclin K is a regulatory subunit of the positive transcription elongation factor b (P-TEFb). P-TEFb is known to positively impact transcription by phosphorylating the large subunit of RNA polymerase II (RNAPII), which fosters the transition toward productive elongation, a critical step in the regulated expression of most genes (144). Whether cyclin K functions similarly to cyclin T in DNA transcription in vivo is debatable (see references in Ref. 204). Western blots indicate that cyclin K protein is downregulated during diapause entry and upregulated upon return to development (204). Knockdown of cyclin K eliminated phosphorylation of RNAPII (at Ser-2 of the COOH-terminal domain of the large subunit). Thus, a case can be made for a potential role of cyclin K in downregulation of transcription during diapause.

Very low rates of protein synthesis also have been demonstrated during diapause in *A. franciscana* embryos (30). The temporal pattern for depression of protein synthesis is quite similar to that seen for the downregulation of both respiration and nucleic acid synthesis. Clearly, the initial cessation of development and cell cycle arrest in diapausing embryos that occur simultaneously with their formation and storage in the ovisac is temporally offset from the metabolic depression, which is only completed many days after embryo release into the water column.

Longevity of macromolecules during diapause. The depression of RNA and protein synthesis during diapause suggests that turnover of macromolecules should be similarly depressed. While considerable literature exists on the stabilization of proteins and mRNA during anoxia-induced quiescence in embryos of *A. franciscana* (3–5, 26, 27, 50, 85, 180, 184, 185), fewer measurements are reported for diapause. Clegg et al. (30) observed little evidence of protein degradation in diapausing embryos and argued that pathways for macromolecular degradation were brought to a standstill during diapause. There are many chaperone-like proteins present at high titer in diapause embryos, most notably p26, artemin, ArHsp21, ArHsp22, and *group 1* and *3* late-embryogenesis abundant (LEA) proteins (28, 82, 102, 155). This suite of proteins likely contributes markedly to the stability of macromolecules in the diapause state. The α-crystallin stress protein p26 has been characterized extensively (e.g., 92, 177, 186, 194). The protein is specifically synthesized in diapause-destined embryos and is degraded after embryonic stages. It possesses chaperone activity (111), and when transfected into mammalian cells, p26 improves desiccation tolerance (113) and inhibits apoptosis during drying and rehydration (186). Artemin is a ferritin homolog that is diapause-specific and exhibits ATP-independent chaperone activity (90, 103). Knockdown studies with artemin indicate that the decrease in artemin extends the time required for release of complete broods of diapausing embryos by the female (103), in addition to reducing the tolerance to desiccation and freezing. Artemin-like and p26-like proteins are expressed in various developmental stages of the freshwater fairy shrimp *Streptocephalus dichotomus* (Anostraca) (132), including the diapausing embryo. Two additional small heat shock proteins (sHSP) have been characterized from *A. franciscana embryos*. ArHSP21 protein is detected in diapause-destined embryos, in-

creases in titer until cyst release, and is absent in second instar larvae and adults (153). ArHSP22 is also synthesized in diapause-destined embryos and degrades during post-diapause development (154). In contrast to the cytoplasmic ArHSP2, ArHSP22 is located in nuclei. Both inhibit heat-induced denaturation of citrate synthase; thus, they likely contribute to protein stability during diapause. Hsp22 was recently shown to be elevated in diapausing copepodids of *Calanus finmarchicus* (9), as was ferritin (181). Finally, numerous LEA proteins from *groups 1* and *3* are expressed in diapausing and post-diapause embryos of *A. franciscana* (16–18, 80, 84, 125, 169, 183, 192, 193). Characterization of these proteins indicates chaperone-like activity and localization to multiple intracellular compartments. In addition to stabilizing proteins against desiccation stress, the *group 3* LEA proteins in *A. franciscana* have been shown recently to stabilize lipid bilayers (131).

A role for pH_i or AMPK? At present, it is unclear whether there exists a regulatory role for acidification of intracellular pH (pH_i) during the entry into diapause in embryos of *A. franciscana*. Previous work has convincingly supported a "pH_i switch" for downregulation of metabolism during anaerobic quiescence in these embryos (21, 22; for reviews, see Refs. 32, 37, 78, 79, 83); pH_i drops rapidly from ≥7.9 (normoxia) to 6.8 within 60 min of anoxic exposure, and further to 6.3 after 24 h. A key and somewhat surprising finding came from ^{31}P-NMR measurements of diapause embryos of *A. franciscana* taken very soon after their release from females, which showed that pH_i was alkaline (≥7.9), i.e., identical to activated post-diapause embryos under normoxia (47). Consequently, it appeared that acidification did not play any role in depression of diapause metabolism. However, as reviewed above, it was later discovered that the severe metabolic depression characterizing *Artemia* diapause is delayed and requires up to 2 wk or more for completion. Clegg and colleagues (28, 31, 32) have made the case that there could be an influence of pH_i acidification on metabolic depression observed during diapause. What is needed are time-course data that follow the pH_i of diapausing embryos across several days after release from the female, so that these values can be compared with the profile for metabolic depression.

AMPK is often considered the metabolic fuel gauge for the cell and acts as a sensitive sensor of cellular energy due to its allosteric stimulation by elevated AMP:ATP ratio, as well as activation through covalent modification (86, 87). When activated, AMPK initiates metabolic and genetic events that restore cellular adenylate status (164). Witt et al. (195) showed that AMPK could be enriched from both diapause and post-diapause embryos of *A. franciscana* and that the enzyme is positively regulated by phosphorylation based on assays of enzyme activity, yet there is no detectable difference in phosphorylation state between the two conditions as judged by Western blots (Witt T, Menze M, and Hand S, unpublished data), a pattern reported by Zhu et al. (207). Thus, despite its apparent role as a master regulator for coordinating numerous catabolic and anabolic pathways, current evidence suggests AMPK is not differentially activated during entry into diapause in *A. franciscana* embryos. Perhaps, it coordinates metabolic processes later in development (refer to *Nematodes*, *AMPK as a downstream effector*).

Environmental cues and other effectors for diapause termination. A thorough review of the descriptive literature on termination cues for crustacean diapause is provided by Lavens and Sorgeloos (107), a review that includes information for anostracans (fairy shrimp, brine shrimp), notostracans (tadpole shrimp), conchostracans (clam shrimp), cladocerans (water fleas), and copepods. Even if one simply focuses on the case for embryos of *A. franciscana*, there are several environmental cues and laboratory treatments that are effective for terminating (breaking) diapause, and they differ depending on the specific population evaluated (31, 46, 107). Environmentally relevant cues include various degrees of desiccation, exposure to low temperature, and exposure to light after drying. Increased levels of CO_2, alkaline-buffered NH_4Cl, oxidants like 3% hydrogen peroxide have been used experimentally in the laboratory to terminate diapause. Combinations of these approaches often enhance their effectiveness. How these agents operate to break diapause in crustaceans is unknown.

Insects

Seeking common mechanisms regulating diapause. Are there mechanisms common to the regulation of diapause in insects? And, might such mechanisms be evident in other animals as well? Perhaps, but the overwhelming observation is that diapause has evolved independently multiple times during the evolutionary history of insects, even within different species in the same genus (e.g., larval, pupal, and adult diapauses all within the genus *Drosophila*, and egg, larval, and adult diapauses in the mosquito genus *Anopheles*). Yet, to achieve a dormant state, such as diapause requires a number of distinct features that likely have a common core.

Although a few insect diapauses are hard-wired and are entered into at a specific developmental stage regardless of the prevailing environmental cues (obligate diapause), the onset of most diapauses in temperate latitudes is programmed by the seasonal pattern of daylength (facultative diapause). Most frequently, the short days of late summer and early autumn provide the environmental cue used by the insect to program its developmental arrest in advance of the upcoming winter. Reliance on this token signal from the seasonal environment enables the insect to anticipate the upcoming unfavorable season, accumulate lipid reserves needed to bridge the many months when food sources are absent, and seek a protected site that will be buffered from the full onslaught of winter. Diapause implies a cell cycle arrest, and depending on the species, can also be accompanied by depression of metabolism, essential for conserving energy reserves. Stress and immune responses are usually elevated during diapause, and mechanisms that protect against cold injury are commonly invoked. How is it that these diverse features are shared by diverse species and in diapauses occurring in different developmental stages? Do these features of the diapause phenotype share a common basis at the level of transcription or do these different diapauses simply converge on the same traits by diverse molecular mechanisms?

Sufficient molecular analyses of diapause have now been completed, allowing us to tentatively suggest that very few transcripts are upregulated in common among different diapausing species. For example, a comparison of diapause upregulated transcripts in the flesh fly *Sarcophaga crassipalpis*

(pupal diapause), *Drosophila melanogaster* (weak adult diapause), and the nematode *Caenorrhabditis elegans* (dauer state) identified only 10 transcripts that were upregulated in common among these three diverse species (157). More closely related species would likely reveal more common expression patterns, but the overriding story is that very few specific transcripts are likely shared across species during diapause. That being said, these different species still generate a similar phenotype, but they do so by different molecular mechanisms. For example, the cell cycle is arrested during diapause in diverse taxa, but how that arrest is achieved differs among species. Arrest in brains of the flesh fly pupae appears to be a consequence of downregulation of proliferating cell nuclear antigen (179), but in several other species, the cell cycle is arrested during diapause by blocking expression of other key cell cycle regulators such as cyclins and cyclin-dependent kinases (178). Thus, with a multitude of inputs that contribute to most features of the diapause syndrome, it is not too surprising that different molecular mechanisms could be used to generate the diapause phenotype.

This component of the review focuses on major molecular pathways that appear to operate in regulating diapause in several insect species. Although the specific check points in the systems may vary with species, the overall involvement of these pathways seems to be common to at least several species. Specifically, this component of the review examines the role of clock genes in programming insect diapause, discusses a recent body of data suggesting a role for insulin/FoxO signaling in generating the downstream phenotype, and identifies a number of missing links whose identity will be essential if we hope to eventually trace the pathway from photoreception to generation of the diapause phenotype.

Sensing the seasonal environment. In temperate latitudes, daylength is a nearly universal seasonal signal used by insects to program the onset of diapause (165, 182). This implies a mechanism to distinguish short days from long days, the ability to store this information within the brain, and then to respond to this acquired information at the appropriate time to bring about an arrest in development. Insects and other animals already have a precise timekeeping mechanism used on a daily basis to maintain daily (circadian) rhythms, and one might very well assume that, with such a mechanism in place, insects would tap into this system for the seasonal clock that distinguishes the annual change in daylength (photoperiodism), an idea proposed by Edwin Bünning many years ago (20).

But, the jury is not completely in on this issue. Experiments showing that null mutants of the clock gene *period* are arrhythmic yet still can enter diapause (166) suggest that this photoperiodic response may, at least in some cases, be operating with a mechanism distinct from the circadian clock (64, 129). But, the independent evolution of diapause in diverse insect groups suggests the possibility that different insect taxa may link their circadian and photoperiodic clocks in different fashions.

Experiments with the bean beetle *Riptortus pedestris* (91) and the mosquito *Culex pipiens* (130) provide some of the strongest evidence that the circadian clock mechanism is essential for this photoperiodic response. In *R. pedestris*, knocking down the clock genes *period* and *cryptochrome2* disrupts both the circadian rhythm of cuticle deposition, as well as the diapause response, causing diapause to be averted in short-day beetles that would normally enter diapause. In contrast, knocking down the clock genes *cycle* or *Clock* causes the opposite effect: beetles programmed by long days to avert diapause enter a diapause-like state. In *Cx. pipiens*, knocking down the negative circadian regulators *period, timeless*, and *cryptochrome2* causes females reared under short-day conditions to avert diapause, while knocking down pigment dispersing factor, a circadian-associated gene, causes the opposite effect, resulting in the diapause phenotype (Fig. 5). Thus, at least for some insect species, it is apparent that an intact and functional circadian clock is essential for eliciting the diapause response. This mechanism is the first critical step in sensing the status of the seasonal environment by evaluating the length of the day and, thereby, determining the correct time to prepare for an overwintering diapause.

How to generate the diverse phenotype of diapause. The next challenge is how to translate the seasonal information on day length into the developmental program we recognize as diapause. Diapause is a complex phenotype comprising traits as diverse as seeking protected habitats, altering food preferences, accumulating fat reserves, halting development, suppressing metabolism, enhancing stress responses, among others. For example, in the mosquito *Cx. pipiens*, exposure to short daylength causes the diapause-destined adult to avoid blood feeding and instead to feed exclusively on nectar sources, seek protection in caves or other buffered underground sites, accumulate huge fat reserves, halt ovarian development, turn on immune response genes, and genes encoding antioxidants, and extend their life span by 9–10 mo, while those reared under long daylength readily seek blood meals from avian hosts, quickly convert those protein-rich meals into eggs, remain active, and then die shortly after laying their eggs, and show a distinctly different profile of gene expression.

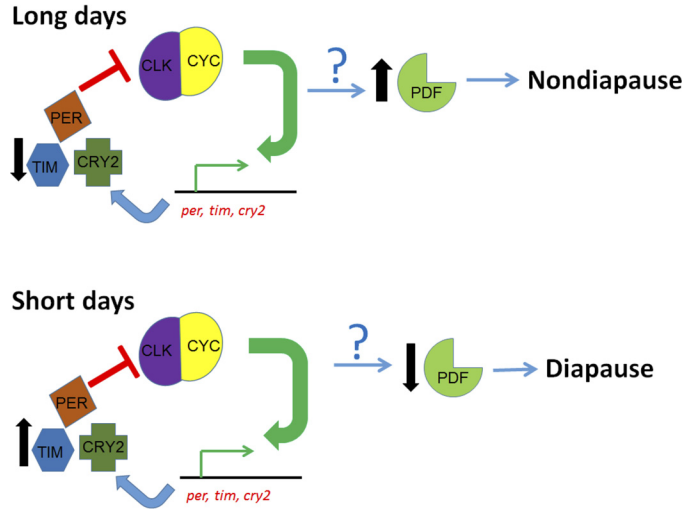

Fig. 5. Knock-down experiments targeting the clock genes shown in this figure suggest that suppression of *period* (*per*), *timeless* (*tim*), or *cryptochrome2* (*cry2*) leads to elevation of *pigment dispersing factor* (*pdf*) and, consequently, the nondiapause phenotype in the mosquito *Culex pipiens*. By contrast, knocking down *pdf* in females reared under long daylengths generates the diapause phenotype. These observations suggest that *per, tim*, and *cry2* transcripts are elevated under short-day, diapause-inducing conditions and that *pdf* is elevated under long day, diapause-averting conditions. Two additional clock elements shown here, *clock* (*clk*) and *cycle* (*cyc*), remain to be tested. [Based on results discussed in Refs. 129 and 130].

Like other cases of adult diapause (44), the diapause of *Cx. pipiens* is prompted by a failure of the corpus allatum to produce juvenile hormone (JH) (158, 176), and this shut-down of the corpus allatum is, at least in part, the consequence of a failure of the brain to synthesize and release allatotropin (95), a neuropeptide that stimulates the corpus allatum to synthesize JH. More recently, this shutdown in ovarian development during diapause was also linked to a shutdown in insulin signaling (IS). Both the IS and JH signaling pathways (Fig. 6) contribute to regulation of the transcription factor, FoxO (170, 171). FoxO, in turn, regulates a diverse network of gene pathways that can result in the many and diverse aspects of the diapause program (173). In *Cx. pipiens*, at least 72 genes have FoxO consensus binding sites, a collection of genes with wide-ranging effects on metabolism, development, cell signaling, transcription, translation, and stress responses. Many of the FoxO targets can be linked to genes with functions that are likely to be important for the success of diapause. Thus far, genes identified and verified as being both FoxO-regulated and upregulated during diapause are linked to overwintering stress tolerance, pathways leading to energy storage and utilization, lifespan extension, cell cycle, development regulation, and a circadian clock gene. Through this single transcription factor it is possible to generate a complex phenotype that has many of the characteristics of diapause.

Certainly, this is not likely to be the only gene pathway to be essential for the diapause phenotype, but the fact that activation of FoxO can trigger numerous downstream genes with functions critical for diapause suggests that it is a major conduit for generating the diverse characteristics that define the diapause syndrome (refer to *Nematodes*, *Histone modifications, small RNAs, microRNAs*).

Unanswered questions. An ambitious, but hopefully attainable, goal for understanding diapause in insects and other organisms is to be able to trace, in detail, the pathway from perception of daylength to the execution of the diapause program. At this time, we have fairly comprehensive insights on several segments of this regulatory cascade: the clock mechanism, the hormonal cues, and a downstream gene pathway (FoxO) that can account for at least some of the diverse manifestations of diapause. But, we are not yet able to link all of these components, especially the clock and the downstream enactment of the hormonal signaling pathways. (e.g., the clock mechanism shown in Fig. 5 and the insulin signaling pathway shown in Fig. 6). How is the environmental information stored in the brain? Diapause is an anticipated response frequently enacted long after the photoperiodic cues have been received. The brain is the site of this storage (43), but how is such information actually stored and then acted upon at a later time during development? Links between the clock mechanism and the downstream signaling pathways essential for diapause are missing and critically needed.

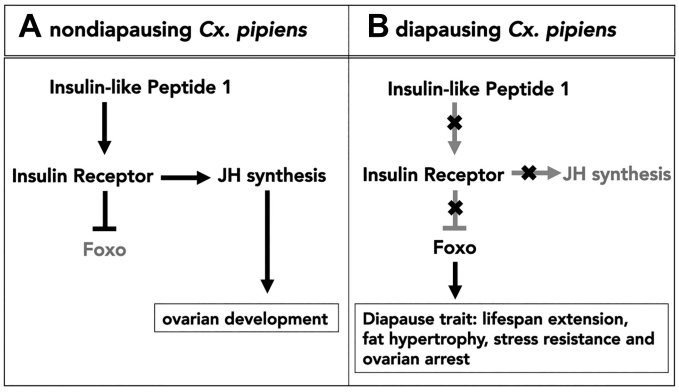

Fig. 6. Model depicting a role for the insulin/FoxO signaling pathway in diapause regulation of the mosquito *Culex pipiens*. *A*: in response to the long daylengths of early summer, insulin signaling leads to juvenile hormone (JH) synthesis and ovarian development and the concurrent suppression of the transcription factor Foxo. *B*: in response to short daylengths of autumn, insulin signaling is shut down, allowing the activation of FoxO, which, in turn, is hypothesized to generate many features of the diapause phenotype. [Based on results discussed in Refs. 170 and 172].

Additional layers of diapause regulation are likely. The environmental inputs (182) and hormonal signals (44) involved in diapause termination are reasonably well understood, yet physiological processes that capacitate the insect to terminate diapause (i.e., the processes of diapause development) remain poorly understood. Cross talk between the brain and fat body in regulating diapause is a dimension only recently recognized (200), and new discoveries in epigenetics (112, 160) portend important revelations for further understanding the complexities of regulating diapause in insects and other animals as well.

Fish

Diversity of vertebrate diapause. Embryonic diapause is not common among the vertebrates, but it is phylogenetically widespread and has almost certainly evolved multiple times (55, 89, 122). In mammals, diapause typically takes the form of delayed implantation at the blastocyst stage (122). Some species of reptiles may arrest development at up to three stages of development: one during early preoviposition development, a second (and most common) during late gastrulation, and perhaps a third in a prehatching embryo (55). Shark diapause occurs in early embryos at the blastodisc stage (190). Among the teleosts, diapause has been reported in autumn-spawning bitterling (98), and in the annual killifishes (197, 198). Mechanistic work on diapause in vertebrate lineages is far less advanced compared with what is known for the invertebrate lineages described above. For practical reasons, most of the physiological and biochemical work on vertebrate diapause has been conducted on embryos of annual killifishes, and thus the rest of this section will focus on advances gained from these studies.

Diapause in the annual killifishes is arguably the best known and most studied among the examples of vertebrate diapause. Arrest of development may occur at three possible stages of development known as diapause I, II, and III (197, 198). Diapause I may occur in pregastrula-stage embryos during a phase of development unique to annual killifishes where the embryonic blastomeres disperse over the yolk in an apparently random fashion prior to reaggregating to form the embryonic axis 4–8 days later (189, 196). Diapause II occurs about midway through development in an embryo that possesses the foundations of the central nervous system and special senses, a functional tubular heart, and a near-full complement of somites (146, 198). Diapause III occurs in the late prehatching embryo in an essentially fully formed precocial larva that has consumed the bulk of the yolk reserves (146, 198). Most studies of diapause in annual killifishes have focused on bioenergetics of

the profound metabolic dormancy and impressive stress tolerance associated with diapause in this lineage (148, 150, 151). Only recently have tools become available to address regulation of diapause and stress tolerance at the molecular level. Below, two aspects of some ongoing work are discussed in which new insights are currently being made with respect to the molecular biology of diapause in annual killifish. First, we discuss a proposed model for the control of entrance into diapause via maternal programming. Second, we address possible molecular mechanisms that support survival and normal development in the face of potentially lethal or teratogenic levels of environmental stress.

Maternal vs. embryonic control of entrance into diapause II. Entrance into diapause II is an alternative developmental pathway in annual killifishes that can be controlled by maternal inputs during oogenesis (61, 145). However, the embryonic incubation environment can alter this initial programming, and, for example, a 5°C increase in incubation temperature can force 100% of the embryos to bypass diapause II and develop directly to diapause III (145). The molecular mechanisms that control maternal influences on diapause II appear to be related to the age of the female at the time of oviposition and not environmental cues, and may be regulated by maternal steroid hormone levels (152). How hormonal cues may alter gene expression during oogenesis, and what exactly is packaged into the oocytes are still open questions. However, evidence is mounting that maternally packaged proteins and perhaps RNAs may play key roles in the maternal regulation of entrance into diapause. Recent evidence from comparative studies of closely related species of killifish that differ in their production of diapause II embryos suggest that microRNAs may play a pivotal role in the regulation of diapause (152). However, mechanistic studies have yet to be reported to support the importance of these findings. On the basis of what we know about the physiology of development and diapause in embryos of the annual killifish, *Austrofundulus limnaeus*, the mechanism for maternal control of entrance into diapause must have the following characteristics: *1*) it must be relatively stable in the developing embryo for at least 10–12 days and through many rounds of cell division, *2*) zygotic signaling mechanisms must be able to interact with and alter the initial programming, and *3*) the system must be responsive to light and temperature experienced by the embryo. Although there are many conceivable routes that would meet all of these requirements, we feel that the maternal packaging of a diapause-specific mRNA is the most likely route. RNAs are known to be specifically packaged into developing oocytes of fish (143) and can be stable for many days postfertilization. In this scenario, environmental exposure during development could lead to production of a specific microRNA to target and silence the expression of the maternally packaged mRNA, similar to the role that mir-430s play in the maternal to zygotic transition in zebrafish (62). This model (Fig. 7) readily allows for maternal provisioning and embryonic alteration of the initial signal and is currently under investigation in embryos of *A. limnaeus* (Romney AL and Podrabsky JE, personal observation). Interestingly, a miR-430 microRNA has been reported to be underexpressed in lineages that produce diapausing embryos, when diapausing embryos are compared with a roughly equivalent stage in nondiapausing embryos (45) (refer to *Nematodes*, *Other resting stages*; *Crustaceans*, *Arrest of the cell cycle, transcription and protein synthesis;* for insects, see *Perspectives and Significance*). While this is an interesting observation that seems to support the mechanism proposed above, there are issues with the experimental design that prevent solid conclusions from being drawn with respect to the role of miR-430 in regulating diapause in annual killifish. First, it may be impossible to find equivalent developmental stages to diapause for comparison in lineages that do not produce diapausing embryos. Second, once embryos enter diapause, the decision has already been made, and thus, the role of a miR-430 in preventing diapause cannot really be evaluated. Lastly, miR-430 family microRNAs are diverse and have many developmental roles. Thus, while these data support a role for miR-430 in the regulation of diapause in annual killifish, further experiments within a species will be needed to confirm such a role.

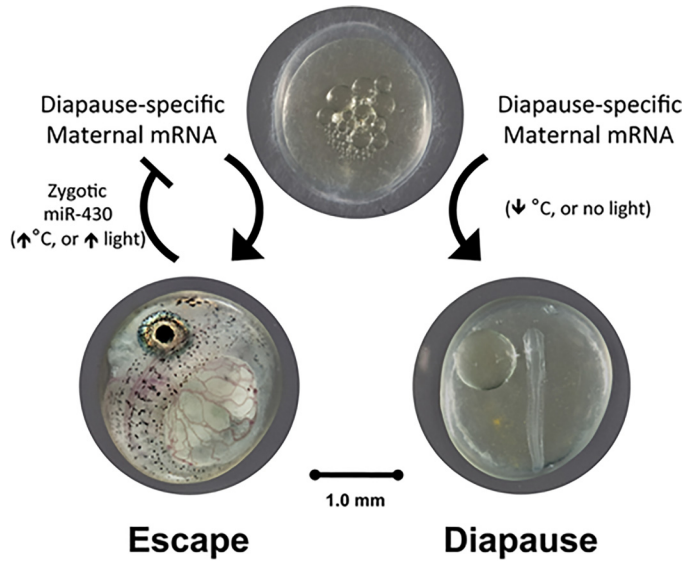

Fig. 7. A proposed model for the environmental alternation of maternally programmed diapause in embryos of the annual killifish *Austrofundulus limnaeus*. In this model, embryos are programmed to enter into diapause II through the provisioning of specific maternal mRNA transcripts during oogenesis. In response to the correct environmental signals, such as increases in incubation temperature or exposure to long-day photoperiods, the developing embryo expresses specific miR-430 microRNAs that clear the maternal mRNAs and favor direct development. Preliminary data suggest overexpression of miR-430 RNAs in embryos that bypass diapause II due to increased incubation temperature (Romney AL and Podrabsky J, personal observation).

Tolerance of environmental stress. Embryonic diapause serves two main ecological purposes. First, it synchronizes the production of young with environmental conditions conducive to survival and growth. Second, in free-living embryos, it often serves to help individuals survive harsh environmental conditions that are lethal or teratogenic to developing embryos. This tolerance of environmental stress is often extreme and sometimes far exceeds conditions that the embryos must actually face in their environment (84). As highlighted above, a great deal of attention has been paid to the accumulation of stabilizing agents during diapause, such as heat shock proteins and other molecular chaperones, as well as small-molecule protectants, such as trehalose (36, 80, 101, 103, 114, 115, 125, 149, 183). These agents undoubtedly help protect and stabilize the cellular macromolecules, membranes, and organelles in dormant embryos. In addition, it appears that avoidance of programmed cell death in response to potentially damaging

stress is also of central importance to survival of dormant embryos (81, 124, 127, 128, 188). However, relatively little attention has been focused on maintenance of genome integrity during embryonic diapause, and how DNA damage may affect survival of dormant embryos. This is an important concept to explore, especially considering that some believe accumulated DNA damage may limit the life span of dormant anhydrobiotic organisms, such as tardigrades (139), despite their ability to withstand rather massive doses of UV radiation (2).

Recently, we have quantified the tolerance of developing and diapausing embryos of *A. limnaeus* to DNA damage caused by UV-C radiation. UV-C radiation induces the formation of cyclobutane pyrimidine dimers and 6-4 pyrimidine-pyrimidone photoproducts (6-4) in DNA that can lead to cell death or cell cycle arrest if not repaired (174, 201). Not surprisingly, diapause II embryos have an extremely high tolerance of UV radiation-induced DNA damage that is 16-fold higher than other fish embryos (188). This tolerance is quite impressive compared with other vertebrates and invertebrates, including dauer larvae from a number of species of nematodes (67). Unfortunately, comparable data for UV radiation tolerance of *C. elegans* dauer larvae do not appear to be available in the literature. In contrast, diapausing embryos of *Artemia* exhibit orders of magnitude higher tolerance of UV light due, in large part, to UV shielding provided by the egg shell (180). Survival of UV radiation in *A. limnaeus* embryos is not supported by accumulation of compounds that shield the embryos from damage, but rather from a high capacity for repair of DNA lesions. If allowed to recover in full-spectrum white light following UV-C irradiation, embryos can effectively and efficiently repair the DNA damage within 48 h (188). However, if kept in the dark, the DNA lesions are not repaired, suggesting an active role for photolyase-mediated DNA repair in these embryos. While survival of diapause II embryos is initially near 100%, even after excessively high doses of UV-C (almost 5,000 J/m^2), after 3 wk in the dark, more than 60% of the embryos perished (Fig. 8; Ref. 188). These data support the conclusion that DNA damage itself does not immediately lead to cell death, but rather the loss of genome integrity eventually leads to failures in gene expression or loss of critical regulatory processes within the cell. Thus, the highly depressed rates of gene expression observed in diapausing embryos (30, 147) and withdrawal from the cell cycle in the G_1 phase (38, 123) may actually contribute to the excessive stress tolerance observed during diapause simply because the DNA is not being actively transcribed or replicated. Eventually, cell damage and death would be caused by errors or interruptions in transcription due to unrepaired DNA damage that impedes the transcriptional machinery. Work in mammalian and yeast model systems supports an intimate connection between DNA damage and areas of active transcription playing a critical role in genome stability and disease progression (1, 77). This model would predict delayed mortality in developing embryos exposed to lethal doses of ultraviolet light in the presence of transcriptional inhibitors; these studies are the logical next step in evaluating the role of transcription in mediating cell damage or death due to DNA damage.

Another fascinating aspect of stress tolerance during diapause in annual killifishes is the ability of the dispersed cell phases of development to act as a buffer against irreparable

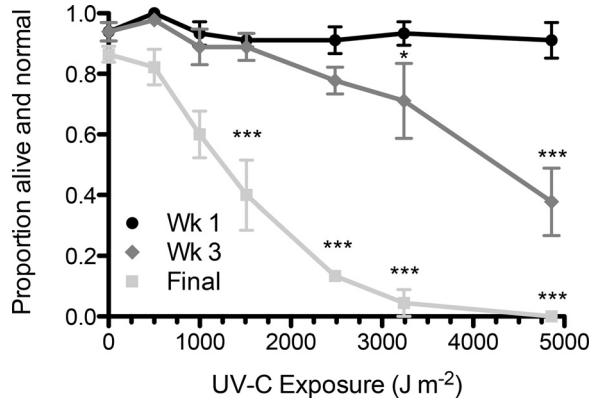

Fig. 8. Initial survival is high in diapause II embryos of *Austrofundulus limnaeus* exposed to UV-C light at 254 nm and allowed to recover in the dark. However, after several weeks, embryos begin to die, and even more embryos die during postdiapause II development after diapause is experimentally broken. Symbols are means ± SE ($n = 3$) *Significantly different from control levels (*$P < 0.05$, ***P ;< 0.001). Wk = weeks of recovery in the dark. Final = final survival of embryos that developed to diapause III 31 days after diapause II was experimentally broken. [Modified from Wagner JT, Podrabsky JE. "Extreme tolerance and developmental buffering of UV-C-induced DNA damage in embryos of the annual killifish Austrofundulus limnaeus."*J Exp Zool A Ecol Genet Physiol* 323A: 10–30, 2015; Published by John Wiley and Sons; Ref. 188].

cellular damage or even cell death. Diapause I can occur during the dispersed cell phases of development, a stage where the embryonic blastomeres are randomly dispersed across the yolk surface. Cell division and proliferation continue during the dispersed cell phase, although cells spend a significant amount of time migrating around the yolk mass (196). The tolerance of these embryos to UV-C-induced DNA damage is higher than in other teleost embryos, but not by a large margin (188). Despite a lack of increased tolerance, UV-C radiation does not cause abnormal development in embryos allowed to recover in the light or the dark, even in embryos exposed to what are known to be teratogenic levels of DNA damage in developing embryos (Fig. 9). Instead, development is delayed for several days, and surviving embryos develop normally. It is not clear at this point whether the delayed development is due to a pause in cell proliferation, as cells repair the damaged DNA, or if the cells are lost and then replaced by cell proliferation. However, there is no evidence for an increase in apoptotic cell death in UV-irradiated embryos (188). Thus, while the tolerance to DNA damage is not high during the dispersed cell phases, mechanisms are in place to allow for normal development despite major damage or cell loses. This ability of dispersed cell phase embryos to incur significant DNA damage and yet avoid abnormal development is unique, and to our knowledge, it is the first example of a developmental stage that is able to buffer against cellular damage or loss during development. Molecular evidence suggests that pluripotency factors, such as oct-4 are expressed in dispersed cells, while expression of genes associated with gastrulation and formation of the embryonic axis is delayed until after the cells reaggregate (189). Thus, an extended period of pluripotency and delay of cellular differentiation may be critical to the ability of dispersed cell phase embryos to develop normally despite receiving potentially teratogenic levels of DNA damage.

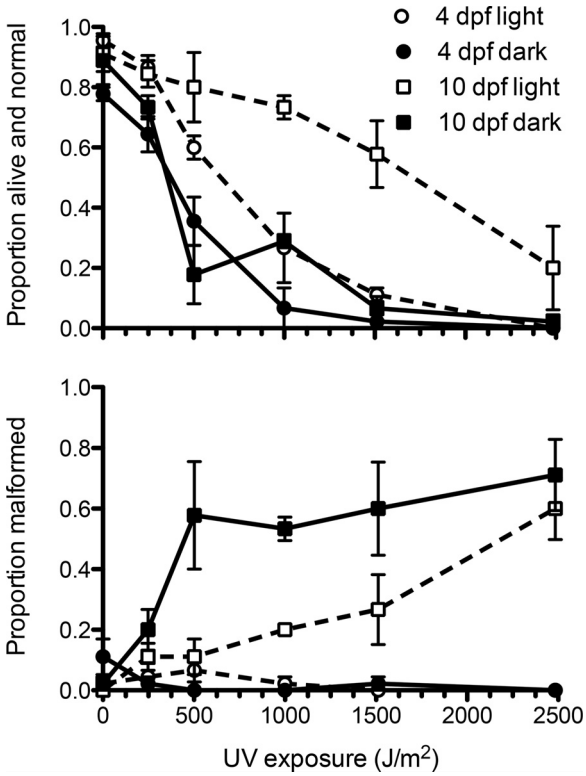

Fig. 9. Survival (*top*) and proportion of abnormal embryos (*bottom*) of *Austrofundulus limnaeus* following exposure to UV-C radiation at 254 nm. Embryos were exposed during the dispersed cell phase (4 dpf) or after formation of the embryonic axis (10 dpf) and allowed to recover in the light or dark. Note the lack of abnormal embryos at 4 dpf, even at levels of irradiation that cause significant teratogenic effects in 10 dpf embryos. [Modified from Wagner JT, Podrabsky JE. "Extreme tolerance and developmental buffering of UV-C-induced DNA damage in embryos of the annual killifish Austrofundulus limnaeus."*J Exp Zool A Ecol Genet Physiol* 323A: 10–30, 2015; Published by John Wiley and Sons; Ref. 188].

Perspectives and Significance

A comprehensive picture of diapause that couples environmental cues to signal transduction and then to the molecular and physiological characteristics of the diapause phenotype is still incomplete. However, new findings for clock genes in insects and their importance in the photoperiodic response that cues diapause are helping to fill gaps. Because of the range of developmental stages across which diapause is expressed and the multiple times the phenotype has evolved across phyla, it is clear that a single suite of mechanisms will not apply to all cases. That is not to say overlapping themes are absent—notably, chromatin/histone modifications in brine shrimp embryos and nematodes, reliance on insulin/FoxO signaling for regulation of gene pathways in *C. elegans* and insects, and cell cycle arrest by cyclin-dependent kinases in some insect species and embryos of the brine shrimp. As with diapause entry, a clear explanation of how diapause is terminated is also incomplete. In crustaceans, it is challenging to conceive of a unifying mechanism that would explain the action of diverse effectors that break diapause like desiccation, cold exposure, oxidants, high CO_2 or NH_4Cl. What is it that these agents have in common? Is each disrupting a key macromolecular interaction that initiates a series of physiological signaling events? For example, knockdown of the small heat shock protein p26 in *Artemia* embryos has demonstrated the importance of this protein in maintaining the diapause state until appropriate cues are received by cysts; in the absence of p26, spontaneous termination of diapause occurs simply after prolonged incubation in seawater (100). As speculated by these authors (100), p26 might bind proteins critical to the maintenance of diapause, or perhaps sequester a signaling protein(s) important for diapause termination. This fundamentally important issue remains an open question.

Preserving the biological integrity of proteins in diapausing animals during long stretches of energy limitation—when protein synthesis required for replacement of macromolecules has been downregulated to conserve energy—is critical for recovery after diapause termination, as are mechanisms for DNA protection and repair. The extensive types of chaperone-like proteins expressed during diapause are major contributors to tolerance to environmental stress during diapause, but more work is needed to clarify the range of their functions and why so many families and isoforms are apparently required (28, 84, 101). Similarly, additional insights about molecular safeguards against unwanted cell death during diapause would be a productive avenue to explore further (81, 124, 126). The dispersed cell stage in embryos of the killifish *A. limnaeus* may provide new insights here (188).

As we enter the exciting new world of microRNAs and other small RNAs, it is also likely that new levels of diapause control will emerge. Several papers already hint at epigenetic contributions to diapause regulation in insects (112, 160), and evidence of cell cycle regulation during diapause by microRNAs has appeared for *A. franciscana* (205). Similar hints are appearing that microRNAs may play a role in regulation of diapause in killifish (45). MicroRNA-mediated regulatory pathways revealed with heterochronic mutants of *C. elegans* are being shown to impact both continuous and interrupted larval development (163). Multiple examples of histone modifications have been reported in *C. elegans* and two species of *Artemia*. It is also likely that there will be a convergence between molecular mechanisms of diapause and mechanisms of lifespan extension. Diapause is, after all, an impressive form of chronological lifespan extension that can extend the duration of an insect's life 10-fold or more. Are common elements shared by diapause and lifespan extension? The building of data sets on these two traits will be instructive in the search for common molecular themes.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: S.C.H., D.L.D., J.E.P., and R.R. prepared figures; S.C.H., D.L.D., J.E.P., and R.R. drafted manuscript; S.C.H., D.L.D., J.E.P., and R.R. edited and revised manuscript; S.C.H., D.L.D., J.E.P., and R.R. approved final version of manuscript.

REFERENCES

1. **Adam S, Polo SE.** Blurring the line between the DNA damage response and transcription: The importance of chromatin dynamics. *Exp Cell Res* 329: 148–153, 2014.
2. **Altiero T, Guidetti R, Caselli V, Cesari M, Rebecchi L.** Ultraviolet radiation tolerance in hydrated and desiccated eutardigrades. *J Zool System Evol Res* 49: 104–110, 2011.
3. **Anchordoguy TJ, Hand SC.** Acute blockage of the ubiquitin-mediated proteolytic pathway during invertebrate quiescence. *Am J Physiol Regul Integr Comp Physiol* 267: R895–R900, 1994.
4. **Anchordoguy TJ, Hand SC.** Reactivation of ubiquitination in Artemia franciscana embryos during recovery from anoxia-induced quiescence. *J Exp Biol* 198: 1299–1305, 1995.
5. **Anchordoguy TJ, Hofmann G, Hand SC.** Extension of enzyme half-life during quiescence in *Artemia* embryos. *Am J Physiol Regul Integr Comp Physiol* 264: R85–R89, 1993.
6. **Angelo G, Van Gilst MR.** Starvation protects germline stem cells and extends reproductive longevity in *C. elegans. Science* 326: 954–958, 2009.
7. **Antebi A, Culotti JG, Hedgecock EM.** daf-12 regulates developmental age and the dauer alternative in *Caenorhabditis elegans*. *Development* 125: 1191–1205, 1998.
8. **Apfeld J, O'Connor G, McDonagh T, DiStefano PS, Curtis R.** The AMP-activated protein kinase AAK-2 links energy levels and insulin-like signals to lifespan in *C. elegans. Genes Dev* 18: 3004–3009, 2004.
9. **Aruda AM, Baumgartner MF, Reitzel AM, Tarrant AM.** Heat shock protein expression during stress and diapause in the marine copepod *Calanus finmarchicus*. *J Insect Physiol* 57: 665–675, 2011.
10. **Bason JV, Runswick MJ, Fearnley IM, Walker JE.** Binding of the inhibitor protein IF_1 to bovine F_1-ATPase. *J. Mol. Biol*. 406, 443–453, 2011.
11. **Baugh LR.** To grow or not to grow: nutritional control of development during *Caenorhabditis elegans* L1 arrest. *Genetics* 194: 539–555, 2013.
12. **Baugh LR, Demodena J, Sternberg PW.** RNA Pol II accumulates at promoters of growth genes during developmental arrest. *Science* 324: 92–94, 2009.
13. **Baugh LR, Sternberg PW.** DAF-16/FOXO regulates transcription of cki-1/Cip/Kip and repression of lin-4 during *C. elegans* L1 arrest. *Curr Biol* 16: 780–785, 2006.
14. **Belovsky GE, Stephens D, Perschon C, Birdsey P, Paul D, Naftz D, Baskin R, Larson C, Mellison C, Luft J, Mosley R, Mahon H, Van Leeuwen J, Allen DV.** The Great Salt Lake ecosystem (Utah, USA): long-term data and a structural equation approach. *Ecosphere* 2: article 33, 2011.
15. **Benesch R.** Zur ontogenie und morphologie von *Artemia salina* L. *Zool Jb (Anat Ontog Tiere)* 86: 307–458, 1969.
16. **Boswell LC, Hand SC.** Intracellular localization of group 3 LEA proteins in embryos of *Artemia franciscana*. *Tissue Cell* 46: 514–519, 2014.
17. **Boswell LC, Moore DS, Hand SC.** Quantification of cellular protein expression and molecular features of group 3 LEA proteins from embryos of *Artemia franciscana*. *Cell Stress Chaperones* 19: 329–341, 2014.
18. **Boswell LC, Menze MA, Hand SC.** Group 3 LEA proteins from embryos of *Artemia franciscana*: Structural properties and protective abilities during desiccation. *Physiol Biochem Zool* 87: 640–651, 2014.
19. **Britton JS, Edgar BA.** Environmental control of the cell cycle in *Drosophila*: nutrition activates mitotic and endoreplicative cells by distinct mechanisms. *Development* 125: 2149–2158, 1998.
20. **Bünning E.** [Die endogene Tagesrhythmik als Grundlage der Photoperiodischen Reaktion]. *Ber Dtsch Bot Ges* 54: 590–607, 1936.
21. **Busa WB, Crowe JH.** Intracellular pH regulates transitions between dormancy and development of brine shrimp (*Artemia salina*) embryos. *Science* 221: 366–368, 1983.
22. **Busa WB, Crowe JH, Matson GB.** Intracellular pH and the metabolic status of dormant and developing *Artemia* embryos. *Arch Biochem Biophys* 216: 711–718, 1982.
23. **Carpenter JF, Hand SC.** Arrestment of carbohydrate-metabolism during anaerobic dormancy and aerobic acidosis in *Artemia* embryos: determination of pH-sensitive control points. *J Comp Physiol B* 156: 451–459, 1986.
24. **Celenza JL, Carlson M.** A yeast gene that is essential for release from glucose repression encodes a protein kinase. *Science* 233: 1175–1180, 1986.
25. **Clegg JS.** Control emergence and metabolism by external osmotic pressure and role of free glycerol in developing cysts of *Artemia salina*. *J Exp Biol* 41: 879–892, 1964.
26. **Clegg JS.** Embryos of *Artemia franciscana* survive four years of continuous anoxia: the case for complete metabolic rate depression. *J Exp Biol* 200: 467–475, 1997.
27. **Clegg JS.** Protein stability in *Artemia* embryos during prolonged anoxia. *Biol Bull* 212: 74–81, 2007.
28. **Clegg JS.** Stress-related proteins compared in diapause and in activated, anoxic encysted embryos of the animal extremophile, *Artemia franciscana*. *J Insect Physiol* 57: 660–664, 2011.
29. **Clegg JS, Conte FP.** A review of the cellular and developmental biology of *Artemia*. In: *The Brine Shrimp Artemia*, edited by Persoone G, Sorgeloos P, Roels O, Jaspers E. Wetteren, Belgium: Universa Press, 1980, p. 11–54.
30. **Clegg JS, Drinkwater LE, Sorgeloos P.** The metabolic status of diapause embryos of *Artemia franciscana* (SFB). *Physiol Zool* 69: 49–66, 1996.
31. **Clegg JS, Jackson SA.** The metabolic status of quiescent and diapause embryos of *Artemia franciscana* (Kellogg). *Archiv für Hydrobiologie Special Issues: Advances in Limnology* 52: 425–439, 1998.
32. **Clegg JS, Trotman CNA.** Physiological, and biochemical aspects of *Artemia* ecology. In: *Artemia: Basic and Applied Biology*, edited by Abatzopoulos ThJ, Beardmore JA, Clegg JS, Sorgeloos and P Dordrecht. Amsterdam, Netherlands: Kluwer Academic, 2002, p. 129–170.
33. **Coburn CM, Mori I, Ohshima Y, Bargmann CI.** A cyclic nucleotide-gated channel inhibits sensory axon outgrowth in larval and adult *Caenorhabditis elegans*: a distinct pathway for maintenance of sensory axon structure. *Development* 125: 249–258, 1998.
34. **Cooley JM.** The effects of temperature on the development of diapausing and subitaneous eggs in several freshwater copepods. *Crustaceana* 35: 27–34, 1978.
35. **Crowe JH, Clegg JS.** *Anhydrobiosis*. Stroudsburg, PA: Dowden, Hutchinson and Ross, 1973.
36. **Crowe JH, Crowe LM, Carpenter JF, Petrelski S, Hoekstra FA, De Araujo P, Panek AD.** Anhydrobiosis: cellular adaptation to extreme dehydration. In: *Handbook of Physiology*, edited by Dantzler W. New York: Oxford University Press, 1997, p. 1445–1477.
37. **Crowe JH, Crowe LM, Drinkwater LE, Busa WB.** Intracellular pH and anhydrobiosis in *Artemia* cysts. In: *Artemia Research and its Applications*, vol. 2, edited by Decleir W, Moens L, Slegers H, Jaspers E, Sorgeloos P. Wetteren, Belgium: Universa Press, 1987, p. 19–40.
38. **Culpepper KM, Podrabsky JE.** Cell cycle regulation during development and dormancy in embryos of the annual killifish *Austrofundulus limnaeus*. *Cell Cycle* 11: 1697–1704, 2012.
39. **Cunningham KA, Bouagnon AD, Barros AG, Lin L, Malard L, Romano-Silva MA, Ashrafi K.** Loss of a neural AMP-activated kinase mimics the effects of elevated serotonin on fat, movement, and hormonal secretions. *PLoS Genet* 10: e1004394, 2014.
40. **Dai JQ, Zhu XJ, Liu FQ, Xiang JH, Nagasawa H, Yang WJ.** Involvement of p90 ribosomal S6 kinase in termination of cell cycle arrest during development of *Artemia*-encysted embryos. *J Biol Chem* 283: 1705–1712, 2008.
41. **Danks HV.** *Insect Dormancy: an Ecological Perspective. Biological* **Survey of Canada** Monograph Series I. Gloucester, Ontario, Canada: Tyrell Press, 1987, 439 p.
42. **Denlinger DL.** Regulation of diapause. *Annu Rev Entomol* 47: 93–122, 2002.
43. **Denlinger DL, Bradfield JY.** Duration of pupal diapause in the tobacco hornworm is determined by number of short days received by the larva. *J Exp Biol* 91: 331–337, 1981.
44. **Denlinger DL, Yocum GD, Rinehart JP.** Hormonal control of diapause. In: *Insect Endocrinology*, edited by Gilbert LI. Amsterdam, Netherlands: Elsevier, 2012, p. 430–463.
45. **Dolfi L, Baumgart M, Groth M, Platzer M, Cellerino AM**MicroRNAs provide the first evidence of genetic link between diapause and aging in vertebrates. *bio$R_x$iv* 017848, 2015.
46. **Drinkwater LE, Clegg JS.** Environmental biology of cyst diapause. In: *Artemia Biology*, edited by Browne RA, Sorgeloos P, Trotman CAN. Boca Raton FL: CRC Press, 1990, p. 93–117.

47. **Drinkwater LE, Crowe JH.** Regulation of embryonic diapause in *Artemia*: Environmental and physiological signals. *J Exp Zool* 241: 297–307, 1987.
48. **Duan RB, Zhang L, Chen DF, Yang F, Yang JS, Yang WJ.** Two p90 ribosomal S6 kinase isoforms are involved in the regulation of mitotic and meiotic arrest in *Artemia*. *J Biol Chem* 289: 16,006–16,015, 2014.
49. **Dutrieu J.** [Observations biochimiques et physiologiques sur le developpement d'*Artemia salina* Leach]. *Arch Zool Exp Gen* 99: 1–133, 1960.
50. **Eads BD, Hand SC.** Mitochondrial mRNA stability and polyadenylation during anoxia-induced quiescence in the brine shrimp *Artemia franciscana*. *J Exp Biol* 206: 3681–3692, 2003.
51. **Elgmork K.** A resting stage without encystment in the annual cycle of the freshwater copepod *Cyclops strenuus*. *Ecology* 36: 739–743, 1955.
52. **Emerson DN.** The metabolism of hatching embryos of the brine shrimp *Artemia salina*. *Proc S D Acad Sci* 42: 131–135, 1963.
53. **Engel M, Hirche HJ.** Seasonal variability and inter-specific differences in hatching of calanoid copepod resting eggs from sediments of the German Bight (North Sea). *J Plankton Res* 26: 1083–1093, 2004.
54. **Euling S, Ambros V.** Reversal of cell fate determination in *Caenorhabditis elegans* vulval development. *Development* 122: 2507–2515, 1996.
55. **Ewert MA.** Cold torpor, diapause, delayed hatching and aestivation in reptiles and birds. In: *Egg Incubation: Its Effects on Embryonic Development in Birds and Reptiles*, edited by Deeming DC, Ferguson MWJ. New York: Cambridge University Press, 1991, p. 173–191.
56. **Faccenda D, Campanella M.** Molecular regulation of the mitochondrial F_1F_o-ATP synthase: physiological and pathological significance of the inhibitory factor 1 (IF_1). *Int J Cell Biol* Article ID 367934, 12 p., 2012.
57. **Fielenbach N, Antebi A.** *C. elegans* dauer formation and the molecular basis of plasticity. *Genes Dev* 22: 2149–2165, 2008.
58. **Fryer G.** Diapause, a potent force in the evolution of freshwater crustaceans. *Hydrobiologia* 320: 1–14, 1996.
59. **Fukuyama M, Rougvie AE, Rothman JH.** *C. elegans* DAF-18/PTEN mediates nutrient-dependent arrest of cell cycle and growth in the germline. *Curr Biol* 16: 773–779, 2006.
60. **Fukuyama M, Sakuma K, Park R, Kasuga H, Nagaya R, Atsumi Y, Shimomura Y, Takahashi S, Kajiho H, Rougvie A, Kontani K, Katada T.** *C. elegans*AMPKs promote survival and arrest germline development during nutrient stress. *Biol Open* 1: 929–936, 2012.
61. **Furness AI, Reznick DN, Springer MS, Meredith RW.** Convergent evolution of alternative developmental trajectories associated with diapause in African and South American killifish. *Proc R Soc B Biol Sci* 282: 20142189, 2015.
62. **Giraldez AJ, Mishima Y, Rihel J, Grocock RJ, Van Dongen S, Inoue K, Enright AJ, Schier AF.** Zebrafish MiR-430 promotes deadenylation and clearance of maternal mRNAs. *Science* 312: 75–79, 2006.
63. **Gledhill JR, Montgomery MG, Leslie AGW, Walker JE.** How the regulatory protein, IF1, inhibits F_1-ATPase from bovine mitochondria. *Proc Natl Acad Sci USA* 104: 15,671–15,676, 2007.
64. **Goto SG, Shiga S, Numata H.** Photoperiodism in insects: perception of light and the role of clock genes. In: *Photoperiodism: The Biological Calendar*, edited by Nelson RJ, Denlinger DL, andSomers DE. New York: Oxford University Press, 2010, p. 258–286.
65. **Green DR, Galluzzi L, Kroemer G.** Metabolic control of cell death. *Science* 345: 1250256, 2014.
66. **Greer EL, Beese-Sims SE, Brookes E, Spadafora R, Zhu Y, Rothbart SB, Aristizábal-Corrales D, Chen S, Badeaux AI, Jin Q, Wang W, Strahl BD, Colaiácovo MP, Shi Y.** A histone methylation network regulates transgenerational epigenetic memory in *C. elegans*. *Cell Rep* 7: 113–126, 2014.
67. **Grewal P, Wang X, Taylor R.** Dauer juvenile longevity and stress tolerance in natural populations of entomopathogenic nematodes: is there a relationship? *Int J Parasitol* 32: 717–725, 2002.
68. **Grice G, Marcus N.** Dormant eggs of marine copepods. *Oceanogr Mar Biol Annu Rev* 19: 125–140, 1981.
69. **Gyllström M, Hansson LA.** Dormancy in freshwater zooplankton: Induction, termination and the importance of benthic-pelagic coupling. *Aquat Sci* 66: 274–295, 2004.
70. **Hahn DA, Denlinger DL.** Energetics of insect diapause. *Annu Rev Entomol* 56: 103–121, 2011.
71. **Hairston NG Jr.** Zooplankton egg banks as biotic reservoirs in changing environments. *Limnol Oceanogr* 41: 1087–1092, 1996.
72. **Hairston NG, Kearns CM.** Temporal dispersal: Ecological and evolutionary aspects of zooplankton egg banks and the role of sediment mixing. *Integr Comp Biol* 42: 481–491, 2002.
73. **Hairston NG Jr, Munns WR Jr.** The timing of copepod diapause as an evolutionarily stable strategy. *Am Nat* 123: 733–751, 1984.
74. **Hairston NG Jr, Olds EJ.** Population differences in the timing of diapause: Adaptation in a spacially heterogeneous environment. *Oecologia (Berl)* 61: 42–48, 1984.
75. **Hall SE, Beverly M, Russ C, Nusbaum C, Sengupta P.** A cellular memory of developmental history generates phenotypic diversity in *C. elegans*. *Curr Biol* 20: 149–155, 2010.
76. **Hall SE, Chirn GW, Lau NC, Sengupta P.** RNAi pathways contribute to developmental history-dependent phenotypic plasticity in *C. elegans*. *RNA* 19: 306–319, 2013.
77. **Hamperl S, Cimprich KA.** The contribution of co-transcriptional RNA: DNA hybrid structures to DNA damage and genome instability. *DNA Rep* 19: 84–94, 2014.
78. **Hand SC.** Metabolic dormancy in aquatic invertebrates. In: *Advances in Comparative and Environmental Physiology*, vol. 8, edited by Gilles R. New York: Springer-Verlag, 1991, p. 1–50.
79. **Hand SC, Hardewig I.** Downregulation of cellular metabolism during environmental stress: Mechanisms and implications. *Annu Rev Physiol* 58: 539–563, 1996.
80. **Hand SC, Jones D, Menze MA, Witt TL.** Life without water: expression of plant LEA genes by an anhydrobiotic arthropod. *J Exp Zool* 307A: 62–66, 2007.
81. **Hand SC, Menze MA.** Mitochondria in energy-limited states: Mechanisms that blunt the signaling of cell death. *J Exp Biol* 211: 1829–1840, 2008.
82. **Hand SC, Menze MA.** Molecular approaches for improving desiccation tolerance: Insights from the brine shrimp *Artemia franciscana*. *Planta* 242: 379–388, 2015.
83. **Hand SC, Menze MA, Borcar A, Patil Y, Covi JA, Reynolds JA, Toner M.** Metabolic restructuring during energy-limited states: Insights from *Artemia franciscana* embryos and other animals. *J Insect Physiol* 57: 584–594, 2011.
84. **Hand SC, Menze MA, Toner M, Boswell L, Moore D.** Expression of LEA proteins during water stress: Not just for plants any more. *Annu Rev Physiol* 73: 115–134, 2011.
85. **Hardewig I, Anchordoguy TJ, Crawford DL, Hand SC.** Nuclear and mitochondria gene expression in developing and quiescent embryos of *Artemia franciscana*. *Mol Cell Biochem* 158: 139–147, 1996.
86. **Hardie DG.** AMP-activated/SNF1 protein kinases: conserved guardians of cellular energy. *Nat Rev Mol Cell Biol* 8: 774–785, 2007.
87. **Hardie DG, Ross FA, Hawley SA.** AMPK: a nutrient and energy sensor that maintains energy homeostasis. *Nat Rev Mol Cell Biol* 13: 251–262, 2012.
88. **Hirche HJ.** Diapause in the marine copepod *Calanus finmarchicus*—a review. *Ophelia* 44, 129–143, 1996.
89. **Hrbek T, Larson A.** The evolution of diapause in the killifish family Rivulidae (Atherinomorpha, Cyprinodontiformes): a molecular phylogenetic and biogeographic perspective. *Evolution* 53: 1200–1216, 1999.
90. **Hu Y, Bojikova-Fournier S, King AM, MacRae TH.** The structural stability and chaperone activity of artemin, a ferritin homologue from diapause-destined *Artemia* embryos, depend on different cysteine residues. *Cell Stress Chap* 16: 133–141, 2011.
91. **Ikeno T, Tanaka S, Numata H, Goto SG.** Photoperiodic diapause under the control of circadian clock genes in an insect. *BMC Biol* 8: 116, 2010.
92. **Jackson SA, Clegg JS.** Ontogeny of low molecular weight stress protein p26 during early development of the brine shrimp, *Artemia franciscana*. *Dev Growth Different* 38: 153–160, 1996.
93. **Jiang X, Yao F, Li X, Jia B, Zhong G, Zhang J, Zou X, Hou L.** Molecular cloning, characterization and expression analysis of the protein arginine *N*-methyltransferase 1 gene (As-PRMT1) from *Artemia sinica*. *Gene* 565: 122–129, 2015.
94. **Jonsson IK.** Causes and consequences of excess resistance in cryptobiotic metazoans. *Physiol Biochem Zool* 76: 429–435, 2003.
95. **Kang DS, Denlinger DL, Sim C.** Suppression of allatotropin simulates reproductive diapause in the mosquito *Culex pipiens*. *J Insect Physiol* 64: 48–53, 2014.
96. **Karp X, Greenwald I.** Control of cell-fate plasticity and maintenance of multipotency by DAF-16/FoxO in quiescent *Caenorhabditis elegans*. *Proc Natl Acad Sci USA* 110: 2181–2186, 2013.
97. **Katz DJ, Edwards TM, Reinke V, Kelly WGA.** *C. elegans* LSD1 demethylase contributes to germline immortality by reprogramming epigenetic memory. *Cell* 137: 308–320, 2009.

98. **Kawamura K, Uehara K.** Effects of temperature on free embryonic diapause in the autumn spawning bitterling *Acheilognathus rhombeus* (Teleostei: Cyprinidae). *J Fish Biol* 67: 684–695, 2005.
99. **Kimura KD, Tissenbaum HA, Liu Y, Ruvkun G.** daf-2, an insulin receptor-like gene that regulates longevity and diapause in *Caenorhabditis elegans*. *Science* 277: 942–946, 1997.
100. **King AM, MacRae TH.** The small heat shock protein p26 aids development of encysting *Artemia* embryos, prevents spontaneous diapause termination and protects against stress. *PLoS One* 7: e43723, 2012.
101. **King AM, MacRae TH.** Insect heat shock proteins during stress and diapause. *Annu Rev Entomol* 60: 59–75, 2015.
102. **King AM, Toxopeus J, MacRae TH.** Functional differentiation of small heat shock proteins in diapause-destined *Artemia* embryos. *FEBS J* 280: 4761–4772, 2013.
103. **King AM, Toxopeus J, MacRae TH.** Artemin, a diapause-specific chaperone, contributes to the stress tolerance of *Artemia franciscana* cysts and influences their release from females. *J Exp Biol* 217: 1719–1724, 2014.
104. **Kroemer G, Galluzzi L, Brenner C.** Mitochondrial membrane permeabilization in cell death. *Physiol Rev* 87: 99–163, 2007.
105. **Krogh A.** The progress of physiology. *Am J Physiol* 90: 243–251, 1929.
106. **Lagha M, Bothma JP, Esposito E, Ng S, Stefanik L, Tsui C, Johnston J, Chen K, Gilmour DS, Zeitlinger J, Levine MS.** Paused Pol II coordinates tissue morphogenesis in the *Drosophila* embryo. *Cell* 23: 976–987, 2013.
107. **Lavens P, Sorgeloos P.** The cryptobiotic state of *Artemia* cysts, its diapause deactivation and hatching; a review. In: *Artemia Research and Its Applications*, vol. 3, edited by Sorgeloos P, Bengtson DA, Decleir W, Jaspers E. Wetteren, Belgium: Universa Press, 1987, p. 27–63.
108. **Lee JH, Koh H, Kim M, Kim Y, Lee SY, Karess RE, Lee SH, Shong M, Kim JM, Kim J, Chung J.** Energy-dependent regulation of cell structure by AMP-activated protein kinase. *Nature* 447: 1017–1020, 2007.
109. **Lees AD.** *Physiology of Diapause in Arthropods*. Cambridge: Cambridge University Press, 1955, 151 p.
110. **Li R, Chen DF, Zhou R, Jia SN, Yang JS, Clegg JS, Yang WJ.** Involvement of polo-like kinase 1 (Plk1) in mitotic arrest by inhibition of mitogen-activated protein kinase-extracellular signal-regulated kinase-ribosomal S6 kinase 1 (MEK-ERK-RSK1) cascade. *J Biol Chem* 287: 15,923–15,934, 2012.
111. **Liang P, Amons R, MacRae TH, Clegg JS.** Purification, structure and molecular chaperone activity in vitro of *Artemia* p26, a small heat shock/a-crystallin protein. *Eur J Biochem* 243: 225–232, 1997.
112. **Lu YX, Denlinger DL, Xu WH.** Polycomb repressive complex 2 (PRC2) protein ESC regulates insect developmental timing by mediating H3K27me3 and activating prothoracicotropic hormone gene expression. *J Biol Chem* 288: 23,554–23,564, 2013.
113. **Ma X, Jamil K, MacRae TH, Clegg JS, Russell JM, Villaneuve TS, Euloch M, Sun Y, Crowe JH, Tablin F, Oliver AE.** A small stress protein acts synergistically with trehalose to confer desiccation tolerance on mammalian cells. *Cryobiology* 51: 15–28, 2005.
114. **MacRae TH.** Diapause: diverse states of developmental and metabolic arrest. *J Biol Res* 3: 3–14, 2005.
115. **MacRae TH.** Gene expression, metabolic regulation and stress tolerance during diapause. *Cell Mol Life Sci* 67: 2405–2424, 2010.
116. **Maps F, Record NR, Pershing AJ.** A metabolic approach to dormancy in pelagic copepods helps explaining inter- and intra-specific variability in life-history strategies. *J Plankton Res* 36: 18–30, 2014.
117. **Marcus NH.** Photoperiodic and temperature regulation of diapause in *Labidocera aestiva* (Copepoda: Calanoida). *Biol Bull* 162: 45–62, 1982.
118. **Marcus NH.** Differences in the duration of egg diapause of *Labidocera aestiva* (Copepoda: Calanoida) from the Woods Hole, Massachusetts, region. *Biol Bull* 173: 169–177, 1987.
119. **Marcus NH.** Ecological and evolutionary significance of resting eggs in marine copepods: Past, present, and future studies. *Hydrobiologia* 320: 141–152, 1996.
120. **Marcus NH, Lutz RV.** Longevity of subitaneous and diapause eggs of *Centropages hamatus* (Copepoda: Calanoida) from the northern Gulf of Mexico. *Mar Biol* 131: 249–257, 1998.
121. **Maxwell CS, Kruesi WS, Core LJ, Kurhanewicz N, Waters CT, Lewarch CL, Antoshechkin I, Lis JT, Meyer BJ, Baugh LR.** Pol II docking and pausing at growth and stress genes in *C. elegans*. *Cell Rep* 6: 455–466, 2014.
122. **Mead RA.** Embryonic diapause in vertebrates. *J Exp Zool* 266: 629–641, 1993.
123. **Meller CL, Meller R, Simon RP, Culpepper KM, Podrabsky JE.** Cell cycle arrest associated with anoxia-induced quiescence, anoxic preconditioning, and embryonic diapause in embryos of the annual killifish *Austrofundulus limnaeus*. *J Comp Physiol B* 182: 909–920, 2012.
124. **Meller CL, Podrabsky JE.** Avoidance of apoptosis in embryonic cells of the annual killifish *Austrofundulus limnaeus* exposed to anoxia. *PLoS One* 8: e75837, 2013.
125. **Menze MA, Boswell L, Toner M, Hand SC.** Occurrence of mitochondria-targeted LEA gene in animals increases organelle resistance to water stress. *J Biol Chem* 284: 10,714–10,719, 2009.
126. **Menze MA, Fortner G, Nag S, Hand SC.** Mechanisms of apoptosis in Crustacea: What conditions induce versus suppress cell death? *Apoptosis* 15: 293–312, 2010.
127. **Menze MA, Hand SC.** Caspase activity during cell stasis: Avoidance of apoptosis in an invertebrate extremophile, *Artemia franciscana*. *Am J Physiol Regul Integr Comp Physiol* 292: R2039–R2047, 2007.
128. **Menze MA, Hutchinson K, Laborde SM, Hand SC.** Mitochondrial permeability transition in the crustacean *Artemia franciscana*: absence of a calcium-regulated pore in the face of profound calcium storage. *Am J Physiol Regul Integr Comp Physiol* 289: R68–R76, 2005.
129. **Meuti ME, Denlinger DL.** Evolutionary links between circadian clocks and photoperiodic diapause in insects. *Integr Comp Biol* 53: 131–143, 2013.
130. **Meuti ME, Stone M, Ikeno T, Denlinger DL.** Functional circadian clock genes are essential for the overwintering diapause of the Northern house mosquito, *Culex pipiens*. *J Exp Biol* 218: 412–422, 2015.
131. **Moore DS, Hansen R, Hand SC.** Liposomes with diverse compositions are protected during desiccation by LEA proteins from *Artemia franciscana* and trehalose. *Biochim Biophys Acta (Biomembranes)* 1858: 104–115, 2016.
132. **Munuswamy N, Sathyanarayanan S, Priyadarshini A.** Embryonic development and occurrence of p26 and artemin-like protein in the cryptobiotic cysts of freshwater fairy shrimp *Streptocephalus dichotomus* Baird. *Curr Sci* 96: 103–110, 2009.
133. **Muramatsu S.** Studies on the physiology of Artemia embryos. 1. Respiration and its main substrate during the early development of the encysted embryo. *Embryologia* 5: 95–106, 1960.
134. **Nakanishi YH, Iwasaki T, Okigaki T, Kato H.** Cytological studies of *Artemia salina*. I. Embryonic development without cell multiplication after the blastula stage in encysted dry eggs. *Annot Zool Jpn* 35: 223–228, 1962.
135. **Nakanishi YH, Okigaki T, Kato H, Iwasaki T.** Cytological studies of *Artemia salina*. II. Deoxyribonucleic acid (DNA) content and the chromosomes in encysted dry eggs and nauplii. *Proc Jpn Acad Sci* 39: 306–309, 1963.
136. **Nambu Z, Tanaka S, Nambu F.** Influence of photoperiod and temperature on reproductive mode in the brine shrimp, *Artemia franciscana*. *J Exp Zool* 301A: 542–546, 2004.
137. **Narbonne P, Roy R.** Inhibition of germline proliferation during *C. elegans* dauer development requires PTEN, LKB1 and AMPK signalling. *Development* 133: 611–619, 2006.
138. **Narbonne P, Roy R.** *Caenorhabditis elegans* dauers need LKB1/AMPK to ration lipid reserves and ensure long-term survival. *Nature* 457: 210–214, 2009.
139. **Neumann S, Reuner A, Brümmer F, Schill RO.** DNA damage in storage cells of anhydrobiotic tardigrades. *Comp Biochem Physiol A Mol Integr Physiol* 153: 425–429, 2009.
140. **Olson C, Clegg J.** Nuclear numbers in encysted dormant embryos of different *Artemia salina* populations. *Experientia* 32: 864–865, 1976.
141. **Olson C, Clegg J.** Cell division during the development of *Artemia salina*. *Wilhelm Roux's Arch* 184, 1–13, 1978.
142. **Patil Y, Marden B, Brand MD, SC.** Hand Metabolic downregulation and inhibition of carbohydrate catabolism during diapause in embryos of *Artemia franciscana*. *Physiol Biochem Zool* 86: 106–118, 2013.
143. **Pelegri F.** Maternal factors in zebrafish development. *Dev Dyn* 228: 535–554, 2003.
144. **Peterlin BM, Price DH.** Controlling the elongation phase of transcription with P-TEFb. *Mol Cell* 23: 297–305, 2006.
145. **Podrabsky JE, Garrett IDF, Kohl ZF.** Alternative developmental pathways associated with diapause regulated by temperature and maternal influences in embryos of the annual killifish *Austrofundulus limnaeus*. *J Exp Biol* 213: 3280–3288, 2010.

146. **Podrabsky JE, Hand SC.** The bioenergetics of embryonic diapause in an annual killifish, *Austrofundulus limnaeus*. *J Exp Biol* 202: 2567–2580, 1999.
147. **Podrabsky JE, Hand SC.** Depression of protein synthesis during diapause in embryos of the annual killifish *Austrofundulus limnaeus*. *Physiol Biochem Zool* 73: 799–808, 2000.
148. **Podrabsky JE, Hand SC.** Physiological strategies during animal diapause: Lessons from brine shrimp and annual killifish. *J Exp Biol* 218: 1897–1906, 2015.
149. **Podrabsky JE, Somero GN.** An inducible 70 kDa-class heat shock protein is constitutively expressed during early development and diapause in the annual killifish *Austrofundulus limnaeus*. *Cell Stress Chaperones* 12: 199–204, 2007.
150. **Podrabsky JE, Tingaud-Sequeira A, Cerdà J.** Metabolic dormancy and responses to environmental desiccation in fish embryos. In: *Dormancy and Resistance in Harsh Environments*, edited by Lubzens E, Cerda J. New York: Springer, 2010, p. 203–226.
151. **Polačik M, Podrabsky JE.** Temporary environments. In: *Extremophile Fishes: Ecology, Evolution, and Physiology of Teleosts in Extreme Environments*, edited by Riesch R, Tobler M, Plath M. New York: Springer, 2015, p. 217–245.
152. **Pri-Tal BM, Blue S, Pau FKY, Podrabsky JE.** Hormonal components of altered developmental pathways in the annual killifish, *Austrofundulus limnaeus*. *Gen Comp Endocrinol* 174: 166–174, 2011.
153. **Qiu ZJ, MacRae TH.** ArHsp21, a developmentally regulated small heat-shock protein synthesized in diapausing embryos of *Artemia franciscana. Biochem J* 411: 605–611, 2008.
154. **Qiu ZJ, MacRae TH.** ArHsp22, a developmentally regulated small heat shock protein produced in diapause-destined *Artemia* embryos, is stress inducible in adults. *FEBS J* 275: 3556–3566, 2008.
155. **Qiu ZJ, MacRae TH.** A molecular overview of diapause in embryos of the crustacean, *Artemia franciscana*. In: *Dormancy and Resistance in Harsh Environments*, Topics in Current Genetics vol. 21, edited by Lubzens E. Berlin: Springer-Verlag, 2010, p. 165–187.
156. **Radzikowski J.** Resistance of dormant stages of planktonic invertebrates to adverse environmental conditions. *J Plankton Res* 35: 707–723, 2013.
157. **Ragland GJ, Denlinger DL, Hahn DA.** Mechanisms of suspended animation are revealed by transcript profiling of diapause in the flesh fly. *Proc Natl Acad Sci USA* 107: 14,909–14,914, 2010.
158. **Readio J, Chen MH, Meola R.** Juvenile hormone biosynthesis in diapausing and nondiapausing *Culex pipiens* (Diptera: Culicidae). *J Med Entomol* 36: 355–360, 1999.
159. **Ren P, Lim CS, Johnsen R, Albert PS, Pilgrim D, Riddle DL.** Control of *C. elegans* larval development by neuronal expression of a TGF-β homolog. *Science* 274: 1389–1391, 1996.
160. **Reynolds JA, Clark J, Diakoff SJ, Denlinger DL.** Transcritional evidence for small RNA regulation of pupal diapause in the fleh fly, *Sarcophaga bullata. Insect Biochem Mol Biol* 43: 982–989, 2013.
161. **Riddle DL.** The Dauer Larva. In: *The Nematode Caenorhabditis elegans*, edited by Wood WB. Cold Spring Harbor, NY: CSHL Press, 1988, p. 393–412.
162. **Riddle DL.** The dauer larva. In: *C. elegans II*, edited by Riddle DL, Blumenthal T, Meyer BJ, Priess JR. Cold Spring Harbor, NY: CSHL Press, 1997, p. 739–768.
163. **Rougvie AE, Moss EG.** Developmental transitions in *C. elegans* larval stages. *Curr Top Dev Biol* 105: 153–180, 2013.
164. **Ruderman NB, Xu XJ, Nelson L, Cacicedo JM, Saha AK, Lan F, Ido Y.** AMPK and SIRT1: a long-standing partnership? *Am J Physiol Endocrinol Metab* 298: E751–E760, 2010.
165. **Saunders D.** *Insect Clock*, 3rd ed., Amsterdam: Elsevier, 2002.
166. **Saunders D, Henrich VC, Gilbert LI.** Induction of diapause in *Drosophila melanogaster:* photoperiodic regulation and the impact of arrhythmic clock mutations on time measurement. *Proc Natl Acad Sci USA* 86: 3748–3752, 1989.
167. **Scheef LP, Marcus NH.** Occurrence and significance of copepod resting egg accumulation in seagrass sediments. *Mar Ecol Prog Ser* 407: 125–134, 2010.
168. **Schindler AJ, Baugh LR, Sherwood DR.** Identification of late larval stage developmental checkpoints in *Caenorhabditis elegans* regulated by insulin/IGF and steroid hormone signaling pathways. *PLoS Genet* 10: e1004426, 2014.
169. **Sharon MA, Kozarova A, Clegg JS, Vacratsis PO, Warner AH.** Characterization of a group 1 late embryogenesis abundant protein in encysted embryos of the brine shrimp *Artemia franciscana*. *Biochem Cell Biol* 87: 415–430, 2009.
170. **Sim C, Denlinger DL.** Insulin signaling and FOXO regulate the overwintering diapause of the mosquito *Culex pipiens. Proc Natl Acad Sci USA* 105: 6777–6781, 2008.
171. **Sim C, Denlinger DL.** Juvenile hormone III suppresses forkhead of transcription factor in the fat body and reduces fat accumulation in the diapausing mosquito *Culex pipiens. Insect Mol Biol* 22: 1–11, 2013.
172. **Sim C, Denlinger DL.** Insulin signaling and the regulation of insect diapause. *Front Physiol* 4: 189, 2013.
173. **Sim C, Kang DS, Kim S, Bai X, Denlinger DL.** Identification of FOXO targets that generate the diverse features of the diapause phenotype in the mosquito *Culex pipiens. Proc Natl Acad Sci USA* 112: 3811–3816, 2015.
174. **Sinha RP, Häder DP.** UV-induced DNA damage and repair: a review. *Photochem Photobiol Sci* 1: 225–236, 2002.
175. **Slegers H.** Enzyme activities through development: a synthesis of the activity and control of the various enzymes as the embryo matures. In: *Artemia Biology*, edited by Browne RA, Sorgeloos P, Trotman CAN. Boca Raton, FL: CRC Press, 1991, p. 37–73.
176. **Spielman A.** Effect of synthetic juvenile hormone on ovarian diapause in *Culex pipiens. Ann Entomol Soc Am* 66: 905–907, 1973.
177. **Sun Y, Mansour M, Crack JA, Gass GL, MacRae TH.** Oligomerization, chaperone activity, and nuclear localization of p26, a small heat shock protein from Artemia franciscana. *J Biol Chem* 279: 39,999–40,006, 2004.
178. **Tammariello SP.** Regulation of the cell cycle during diapause. In: *Insect Timing: Circadian Rhythmicity to Seasonality*, edited by Denlinger DL, Giebultowicz JM, Saunders DS. Amsterdam: Elsevier, 2001, p. 173–183.
179. **Tammariello SP, Denlinger DL.** G_0/G_1 cell cycle arrest in the brain of *Sarcophaga crassipalpis* during pupal diapauses and the expression pattern of the cell cycle regulator, proliferating cell nuclear antigen. *Insect Biochem Mol Biol* 28: 83–89, 1998.
180. **Tanguay JA, Reyes RC, Clegg JS.** Habitat diversity and adaptation to environmental stress in encysted embryos of the crustacean *Artemia. J Biosci* 29: 489–501, 2004.
181. **Tarrant AM, Baumgartner MF, Verslycke1 T, Johnson CL.** Differential gene expression in diapausing and active *Calanus finmarchicus* (Copepoda). *Mar Ecol Prog Ser* 355: 193–207, 2008.
182. **Tauber MJ, Tauber CA, Masaki S.** *Seasonal Adaptations of Insects*. New York: Oxford University Press, 1986.
183. **Toxopeus J, Warner AH, MacRae TH.** Group 1 LEA proteins contribute to the desiccation and freeze tolerance of *Artemia franciscana* embryos during diapause. *Cell Stress Chaperones* 19: 939–948, 2014.
184. **van Breukelen F, Hand SC.** Characterization of ATP-dependent proteolysis in embryos of the brine shrimp, *Artemia franciscana*. *J Comp Physiol B* 170: 125–133, 2000.
185. **van Breukelen F, Maier R, Hand SC.** Depression of nuclear transcription and extension of mRNA half-life under anoxia in *Artemia franciscana* embryos. *J Exp Biol* 203: 1123–1130, 2000.
186. **Villenueve TS, Ma XC, Sun Y, Oulton MM, Oliver AE, MacRae TH.** Inhibition of apoptosis by p26: implications for small heat shock protein function during *Artemia* development. *Cell Stress Chap* 11: 71–80, 2006.
187. **Viollet B, Andreelli F, Jørgensen SB, Perrin C, Flamez D, Mu J, Wojtaszewski JF, Schuit FC, Birnbaum M, Richter E, Burcelin R, Vaulont S.** Physiological role of AMP-activated protein kinase (AMPK): insights from knockout mouse models. *Biochem Soc Trans* 31: 216–219, 2003.
188. **Wagner JT, Podrabsky JE.** Extreme tolerance and developmental buffering of UV-C induced DNA damage in embryos of the annual killifish *Austrofundulus limnaeus*. *J Exp Zool* 323A: 10–30, 2015.
189. **Wagner JT, Podrabsky JE.** Gene expression patterns that support novel developmental stress buffering in embryos of the annual killifish *Austrofundulus limnaeus*. *EvoDevo* 6: 2, 2015.
190. **Waltrick D, Awruch C, Simpfendorfer C.** Embryonic diapause in the elasmobranchs. *Rev Fish Biol Fisheries* 22: 849–859, 2012.
191. **Wang Z, Stoltzfus J, You YJ, Ranjit N, Tang H, Xie Y, Lok JB, Mangelsdorf DJ, Kliewer SA.** The nuclear receptor DAF-12 regulates nutrient metabolism and reproductive growth in nematodes. *PLoS Genet* 11: e1005027, 2015.
192. **Warner AH, Chakrabortee S, Tunnacliffe A, Clegg JS.** Complexity of the heat-soluble LEA proteome in *Artemia* species. *Comp Biochem Physiol D Genom Proteom* 7: 260–267, 2012.
193. **Warner AH, Miroshnychenko O, Kozarova A, Vacratsis PO, MacRae TH, Kim J, Clegg JS.** Evidence for multiple group 1 late embryo-

genesis abundant proteins in encysted embryos of *Artemia* and their organelles. *J Biochem* 148: 581–592, 2010.
194. **Willsie JK, Clegg JS.** Nuclear p26, a small heat shock/alpha-crystallin protein, and its relationship to stress resistance in *Artemia franciscana* embryos. *J Exp Biol* 204: 2339–2350, 2001.
195. **Witt TL, Menze MA, Hand SC.** Isolation and characterization of AMP-activated protein kinase from embryos of *Artemia franciscana*. *Integr Comp Biol* 45: 1210, 2005.
196. **Wourms JP.** The developmental biology of annual fish II. Naturally occuring dispersion and reaggregation of blastomeres during the development of annual fish eggs. *J Exp Zool* 182: 169–200, 1972.
197. **Wourms JP.** Developmental biology of annual fishes I. Stages in the normal development of *Austrofundulus myersi* Dahl. *J Exp Zool* 182: 143–168, 1972.
198. **Wourms JP.** The developmental biology of annual fishes III. Pre-embryonic and embryonic diapause of variable duration in the eggs of annual fishes. *J Exp Zool* 182: 389–414, 1972.
199. **Wurtsbaugh WA, Gliwicz ZM.** Limnological control of brine shrimp population dynamics and cyst production in the Great Salt Lake, Utah. *Hydrobiologia* 466: 1191132, 2001.
200. **Xu WH, Lu YX, Denlinger DL.** Cross-talk between the fat body and brain regulates insect developmental arrest. *Proc Natl Acad Sci USA* 109: 14,687–14,692, 2012.
201. **Yabu T, Todoriki S, Yamashita M.** Stress-induced apoptosis by heat shock, UV and γ-ray irradiation in zebrafish embryos detected by increased caspase activity and whole-mount TUNEL staining. *Fish Sci* 67: 333–340, 2001.
202. **Yang F, Su Chen S, Dai ZM, Chen DF, Duan RB, Wang HL, Jia SN, Yang WJ.** Regulation of trehalase expression inhibits apoptosis in diapause cysts of *Artemia*. *Biochem J* 456: 185–194, 2013.
203. **Zhang X, Yalcin S, Lee DF, Yeh TY, Lee SM, Su J, Mungamuri SK, Rimmelé P, Kennedy M, Sellers R, Landthaler M, Tuschl T, Chi NW, Lemischka I, Keller G, Ghaffari S.** FOXO1 is an essential regulator of pluripotency in human embryonic stem cells. *Nat Cell Biol* 13: 1092–1099, 2011.
204. **Zhao Y, Ding X, Ye X, Zhong-Min Dai Z-M, Yang J-S, Yang W-J.** Involvement of cyclin k posttranscriptional regulation in the formation of *Artemia diapause* cysts. *PLoS One* 7: e32129, 2012.
205. **Zhao LL, Jin F, Ye X, Zhu L, Yang JS, Yang WJ.** Expression profiles of miRNAs and involvement of mir-100 and mir-34 in regulation of cell cycle arrest in *Artemia*. *Biochem J* 470: 223–231, 2015.
206. **Zhou R, Yang F, Chen DF, Sun YX, Yang JS, Yang WJ.** Acetylation of chromatin-associated histone H3 lysine 56 inhibits the development of encysted *Artemia* embryos. *PLoS One* 8: e68374, 2013.
207. **Zhu XJ, Dai JQ, Tan X, Zhao Y, Yang WJ.** Activation of an AMP-activated protein kinase is involved in post-diapause development of *Artemia franciscana*-encysted embryos. *BMC Dev Biol* 9: 21, 2009.