Environmental and Genetic Regulation of the Diapause Termination in Insects[*]

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Abstract Diapause is one of the chief physiological mechanisms by which insects can survive under the adverse environmental conditions. The diapause termination is a special stage for diapause development and characterization. An understanding of the mechanisms underlying diapause termination is critical in the management of any agroecosystem. Numerous studies report that diapause termination can be influenced by many environmental and genetic factors but not summarized. This paper firstly provides an overview of the effect of environmental factors on diapause termination including the low temperature or chilling can prompt diapause termination the change of photoperiod also can terminate most of the summer diapause humidity and food factors can separately or jointly terminate the summer or winter diapause in many insects. Besides the environmental effect then a further discussion is made on the role that the three different kinds of endocrine hormones ecdysteroids or ecdysteroid antagonist juvenile hormones and diapause hormone play in diapause regulation respectively. Finally it is discussed that the molecular mechanisms which may be involved in the process of termination and the problems which had been encountered in the course of recent study on diapause termination such as how to ascertain whether the diapause is terminated with aim to offer a rich potential for contributing not only to an understanding of the molecular basis of insect seasonality but also to other related and timely topics in the agricultural and biomedical sciences.

Key words diapause termination regulation temperature photoperiod food hormone

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The capacity for diapause is widespread among insects and has undoubtedly contributed to the enormous success of the Class Insecta by allowing them to exploit seasonal resources and bridge harsh winters dry seasons or other seasonally inimical conditions [1] . Diapause is not just a cessation of normal development but a dynamic alternate developmental pathway that is characterized by several physiologically distinct states including diapause induction maintenance and termination [2] . The transition from diapause maintenance to termination is typically associated with changes in the insect′s endocrinemilieu [3] . However the physiological and molecular mechanisms that translate ecologically relevant environmental cues into endocrine signaling cascades at diapause termination are poorly understood.

It has been recognized that diapause can be terminated through at least two processes. In natural conditions diapause is usually terminated through a slow process called′ horotelic′ evolving at a standard rate [4]   termination Fig. 1 line A . For example low temperature during the winter is a well-known factor that promotes horotelic termination although low temperature is not an absolute prerequisite for this process [5] . Besides the horotelic process typically under laboratory conditions diapause can be rapidly terminated by a process called′ activation′ or′ tachytelic′ evolving at a faster rate [4]   termination Fig. 1 line B . In most studies tachytelic termination is induced in groups of individuals in a coordinated man-

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ner either by dramatically manipulating the environ-ment or by application of pharmacological agents such as hormones or their analogues [6] . In many other spe-cies however the requirement for specific diapause-terminating conditions [7] is strict and/or fitness is considerably compromised without their intervening effect.

A′ horotelic′ natural termination
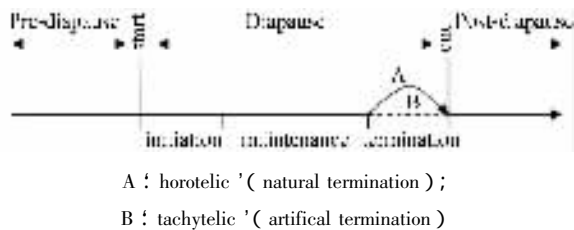
B′ tachytelic′ artifical termination

Fig. 1   Two approaches to diapause termination

Based on the crucial knowledge obtained from ear-lier studies this review makes a brief summary from environmental and genetic control on the diapause ter-mination aiming to summarize the knowledge on the physiological mechanisms underlying diapause termina-tion and also to provide with insight on the molecular basis of insect seasonality.

1 Environmental regulation of dia-pause termination

The termination of diapause in insects is stimula-ted by a number of enviromental components. Among these environmental cues temperature photoperiod moisture and food are often examined in many studies though some other factors may have effect on the dia-pause termination.

1.1 Temperature

Temperature is one of the key environmental stim-uli controlling the termination of diapause. In many in-sects in temperature regions diapause development is promoted and completed by low temperature and mor-phogenesis then resumes when temperature rise so that hatch or emergence are synchronized in spring. Lees [8] has suggested that low temperature is the normal stimu-lus required to terminate diapause under field condi-tions based on the results of Roubaud [9] in which a low temperature causes prompt pupariation after retur-ning to high temperature in L. sericata. There are two contradictory results in L. sericata Ring [10] has shown

a prominent effect of low temperature on diapause ter-mination although Fraser and Smith [11] have observed little effect. In the onion maggot completion of dia-pause occurred at a wide range of temperatures 4 ~ 25 ℃ The optimal temperature was approximately 16 ℃ at which temperature only five days were re-quired for diapause completion [12] . In diapausing sor-ghum midge temperatures in the range from 20 to 30 ℃ were optimum for diapause termination and adult emer-gence moisture acted to initiate diapause termination but photoperiod had no significant effect on the termi-nation of larval diapause [13] .

Chilling is the primary means for diapause termi-nation in many winter diapausing insect species in the field [14] . Tanno [15] found that freezing formation of ice crystals was a necessary factor for termination of prepupal diapause in the field and laboratory. In twig borer Anarsia lineatella Cold storage for at least 30 days at 4 ℃ results in a synchronized reactivation of the larvae with the developmental time of larvae chilled for 45 and 60 days at 4 ℃ becoming significant-ly shorter. The actual mechanisms of chilling treatment on termination of the diapause stage are not clear. However recent studies have suggested that chilling may activate certain gene expressions in diapausing eggs and lead to initiation of diapause termination [16] .

Though there are more prevailing compared with high temperature the requirement of low temperature for the completion of diapause development has been overgeneralized. In many species diapause terminates spontaneously under the conditions above a lower ther-mal threshold in L. sericata. Larval diapause terminated spontaneously when kept continously under the dia-pause-inducing condition of 20 ℃ and L: D = 12: 12 although pupariation was not synchronized [17] . In some other insects diapause can be terminated by transfe-ring to higher temperature [14] . For example a transfer of diapause larvae in C. vicina from 5 ℃ to 20 ℃ or 25 ℃ [5] in L. sericata from 7. 5 ℃ to 25 ℃ or from 20 ℃ to 25 ℃ without changing the photoperiod in-duce prompt and synchronous pupariation. However an increase in temperature is inevitable for the resump-tion of morphogenesis after exposure to a low tempera-

ture therefore it is difficult to distinguish the effects of high temperature in the later period from the effects of the increase in temperature.

1.2 Photoperiod

In all the physical factors that change seasonally photoperiod is the most regular and reliable cue to seasons. Insects have evolved numerous ways to utilize photoperiod as a diapause-regulating cue. If an insect relies upon photoperiodic information for terminating its diapause one might logically assume that the clock system underlying termination of diapause is the same mechanism as the one involved in induction of diapause. Stross [18] shows that light is essential for termination of diapause in the laboratory-cultured strain regardless of the temperature or duration of ephippia storage. Most of the summer diapause and perhaps even some rare case of winter diapause are terminated in the field by the change of photoperiodic signal [19]. For example in the univoltine saturniid *Antheraea yamamai* estival diapause in the pupae was induced when the larvae experienced long days L: D = 16: 8 transfer of diapausing pupae to short daylengths terminated diapause within a few days [20]. In the *Pseudopidorus fasciata* long days terminate diapause with a critical daylength of 14 h [21]. It has been shown in many temperature species with winter diapause that long-day conditions terminate diapause in the laboratory. In most of these species however insects usually show a progressive loss in their sensitivity to photoperiod as diapause development proceeds and by the time of midwinter photoperiodic sensitivity has ceased under natural conditions.

The present studies indicate that the constant darkness DD can facilitate diapause termination. One of the environmental cues for *Artemia* embryonic diapause termination EDT the nature of which is barely understood is the absence of light although light-dark cycles photoperiod have been examined in inducing embryonic diapause. The influence of constant darkness DD on EDT was investigated in laboratory-produced cysts of *Artemia franciscana*. All the DD conditions significantly promoted EDT. Either the plateau value of the percent hatch increased in re-

sponse to each DD condition or the rearing period to attain the half value of the plateau value became shorter [22].

1.3 Water as prerequisite for development

The complexity of the decision about the moment of diapause end increases when water or moisture is a prerequisite for the resumption of development. Often water is considered as essential for diapause completion. In many cases diapause persists for 9 ~ 10 months and during this long period a diapausing egg or pupa has no ability to replenish its water supply by drinking or feeding. This dilemma is further exacerbated by the large surface area volume ratio characteristic of most insects. The beginning of the rainy season means the return of the supply of water in the soil necessary for embryonic development and egg hatching. Water acts as a second factor influencing the end of dormancy of quiescent eggs present in the soil waiting for favorable conditions to resume development [23-24]. Beck [25] has found that in *O. nubilalis* the neuroendocrine system is not activated until after the water requirement has been met. Since the activation of the neuroendocrine system and the secretion of prothoracicotropic hormone is taken as the first postdiapause physiological event the lack of body water prevents the completion of diapause development. Contact with water is reported to serve as the terminating factor for summer diapause of the larvae of the stem borer *Busseola fusca* [26] and of the eggs of the chrysomelid beetle *Homichloda barkeri* [27]. In some crustaceans drying of the sediment increases emergence from diapausing eggs upon re-hydration [28].

1.4 Food host-plant availability

In some insects food is critical for termination of diapause the availability of food may be profoundly influenced by seasonal rhythms [29]. Rains stimulate an increase in plant growth which provides a wealth of new food resources for many phytophagous insects. It′s reported that the end of diapause is related to feeding In many bruchid species the nutritional factors can often be important in the termination for diapause the genesis is stimulated by adult females feeding on the pollen of the holt plant [30].

2 Endocrine regulation of diapause termination

Besides the environmental effect the endocrine system also plays an important role in diapause regulation. Several key hormones serve as regulators of diapause but precisely which hormones are involved depends on the species and the developmental stage in which diapause occurs [31]. In most cases ecdysteroids juvenile hormone JH and diapause hormone DH are involved in its regulation [32].

2.1 Ecdysteroids 20-hydroxyecdysone or ecdysteroid antagonist

Ecdysteroids appear to participate in various events during embryonic development including gastrulation [33] germ band retraction head involution [34] and cuticle formation [35]. The possible role of ecdysteroids in the control of embryonic diapause has also been suggested in several insects. Application of 20-hydroxyecdysone ecdysteroid hormone terminated diapause in eggs of the silkworm *Bombyx mori* [36] *Locusta migratoria* [37] and the cochineal *Lepidosaphes ulmi* [38]. These results imply that embryonic diapause might be regarded as an ecdysteroid-deficiency syndrome. Although many authors have investigated the hormonal control of embryonic development for grasshoppers there has been no report showing that application of ecdysteroids is effective in diapause termination. Besides there is ample evidence that pupal diapause is caused by lack of ecdysteroids and is terminated when the prothoracic glands are activated to ecdysteroid production [39-40]. For example diapausing pupae in the bollworm *Helicoverpa armigera* can be broken by the injection of 20-hydroxyecdysterone [40].

KK-42 as an anti-juvenile hormone JH agent [41] or as ecdysteroid antagonist [42-43] can avert diapause when applied to prediapausing pharate first instar larvae in the gypsy moth but this effect can be countered by application of 20-hydroxyecdysone or the ecdysteroid agonist RH-5992 [44-45]. It is also reported that KK-42 can induce a precocious termination of diapause in pharate first instar larvae of the wild silkmoth *Antheraea yamamai* [46].

2.2 Juvenile hormones JHs

In larval diapause a high juvenile hormone JH title in the hemolymph is reported to be involved in suppression of the brain-prothoracic glands axis preventing the release of ecdysteroids for larval growth and pupation [31]. In fact removal of corpora allata CA from diapausing larvae causes a decrease in JH concentration which induces an increase in hemolymph ecdysteroid thus terminating diapause [47].

A stimulatory effect of JH or JHA on the prothoracic glands at the last larval instar or the pupal stage has been reported in several lepidopteran insects [48-49]. In the noctuid moth *Mamestra brassicae* JH treatment can prevent pupal diapause from stimulating the ecdysone production by the prothoracic glands [50]. In larvae of the bamboo borer *Omphisa fuscidentalis* the hemolymph ecdysteroid concentration is low during diapause [51] and application of JHA stimulates the prothoracic glands to secrete ecdysteroids resulting in the termination of diapause [51]. A stimulatory effect of JH on prothoracic glands was first demonstrated in diapausing pupae of *Hyalophora cecropia* [52]. Visscher inferred from histological observations that JHA stimulates the prothoracic glands to release ecdysteroids [53]. Meantime the topical application of juvenoid can also terminate adult reproductive diapause in many species including *Leptinotarsa decemlineata* [54] and *Aulacophora. nigripennis Coleoptera Chrysomelidae* [53].

2.3 Diapause hormone DH

Diapause hormone the regulator of the embryonic diapause in *B. mori* is a 24-amino acid peptide released from the suboesophageal ganglion of the female to elicit embryonic diapause in her progeny [54]. The cDNA encodes a polyprotein precursor from which DH pheromone biosynthesis-activating neuropeptide and three other neuropeptides are cleaved [55]. The DH-pheromone biosynthesis-activating neuropeptide DH-PBAN gene consists of 6 exons and 5 introns and expression can be noted in 12 neurosecretory cells within the subesophageal ganglion. FXPRL amide peptides encoded by the DH-PBAN gene induce embryonic diapause in *B. mori* but terminate pupal diapause in *Helicoverpa armigera Har* [56]. Ecdysteroidogenesis in prothoracic glands PGs is stimulated by synthetic Har-DH in vivo and in vitro and labeled Har-DH

bound to the membrane of the PG　thus suggesting that DH breaks diapause by activating the PG to synthesize ecdysone. Furthermore　the response of DH in terminating diapause is temperature dependent.

3　Molecular regulation of diapause termination

　　Environmental and hormonal regulators of diapause have been reasonably well defined　but the understanding of the molecular regulation of diapause remains in its infancy. Some genes are known to start their expression only after passage of a specific time-period of diapause and their products may thus participate in the processes of diapause termination [57].

　　In a long series of papers on the cold termination of diapause in the eggs of the silkworm *B. mori*　the physiological mechanism of this process is attributed to the conformational change of two specific proteins. One is named Time-Interval-Measuring-Enzyme TIME　and regulated by the time-holding peptide　PIN　[58]. PIN peptide binds with TIME-EA4 protein to inhibit the activation of ATPase and consequently to regulate time measurement by TIME-EA4 [59]. The other is extracellular signalregulated kinase ERK　a member of mitogen-activated protein kinase　MAPK　which is involved in a variety of cellular events such as cancer cell proliferation　differentiation and stress responses in animals and plants. In *B. mori*　the phosphorylated　activated　ERK decreases during diapause induction but is increased by cold treatment. It's reported that phosphorylation of ERK is increased either by chilling or JHA treatment. No morphological change is observed within 2 days after JHA treatments　but ERK begins to increase in 12 h. Possibly ERK plays a role in diapause termination in *Locusta migratoria*. In pupae of *Manduca sexta*　ERK in the prothoracic glands is activated by PTTH in vitro　and inhibition of ERK by a specific inhibitor　U0126　suppresses ecdysteroid secretion [60]. In diapause eggs of *Bombyx mori*　ERK is activated by either cold exposure or dechorionation each of which has been known to terminate diapause in this moth [61].

　　The present studies demonstrate that COX1　one of the cytochrome *c* oxidase molecule subunits　may be involved in triggering the mechanism that terminates diapause [62]. Shirai [63] has found that induction of cytochrome *c* oxidase in the brain contributes to enhancing ATP production and possibly the subsequent stimulation of PTTH release in the brain. It is not yet clear whether cytochrome *c* oxidase in the brain functions as a key up-regulated enzyme which leads to diapause termination or it is a downstream event.

4　Summary

　　Despite a great amount of detailed studies on many insect species　the understanding of how diapause ends has remained still rather incomplete. To ascertain whether the diapause is terminated　sometimes　it is had to rely on the events of post-diapause development which can be recorded only after exposure to permissive environmental conditions　i. e.　after a shorter or longer delay after the end of diapause. Also　as Hodek [64] has stated　it will be advisable to define clearly whether the end of diapause is identified with the resumption of covert development potential or with the resumption of overt development. Besides　there are not enough studies to show the physiological differences between horotelic termination and tachytelic termination.

　　The early molecular events that prompt the termination of diapause are poorly known but it should prove to be of considerable interest in probing the signaling cascade that leads to the activation of the gene sets needed to initiate morphogenesis or reproduction. Prothoracicotropic hormone and other hormones are known to orchestrate the downstream events　but the critical questions must focus on the events within the brain that prompt the release of these hormones at a certain time thus releasing the insect from its developmental arrest.

　　As a problem in development　diapause offers a unique opportunity to probe regulatory mechanisms that sequentially halt and reinitiate development in whole organisms and in cells　working on diapause also has the potential to offer interesting insights on the molecular basis for aging. Diapause is　in fact　a dramatic example of extending the life span　and much of the recent literature onaging. Future diapause research offers a rich potential for contributing not only to an understanding of the molecular basis of insect seasonality but

also to other related and timely topics in the agricultur-
al and biomedical sciences.

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