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Differential expression of two small Hsps during diapause in the corn stalk borer *Sesamia nonagrioides* (Lef.)

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ABSTRACT

We isolated and characterized two members of the α-crystallin/sHsp family, *SnoHsp19.5* and *SnoHsp20.8* from *Sesamia nonagrioides* (Lepidoptera: Noctuidae). The cDNAs encoded proteins of 174 and 185 amino acids, with calculated molecular weights of 19.5 and 20.8 kDa, respectively. The deduced amino acid sequences of *SnoHsp19.5* and *SnoHsp20.8* showed highest homology to *Hsp19.7* of *Mamestra brassicae* and to *Bombyx mori Hsp20.4*, respectively. Expression patterns of *SnoHsp19.5* and *SnoHsp20.8* in non-diapausing individuals under different environmental conditions (heat or cold) showed different accumulation profiles for the two genes after heat and cold treatment. *SnoHsp19.5* was consistently expressed, while *SnoHsp20.8* gene was down-regulated in deep diapause and was up-regulated at the termination of diapause. Our results suggest that these two genes play distinctive roles in the regulation of diapause.

1. Introduction

In response to heat and other stressors, nearly all organisms express heat shock proteins (Hsps). Hsps are conserved proteins involved in multiple cellular processes, such as protein folding, acting as molecular chaperons and most are up-regulated following stress (Hartl and Hayer-Hartl, 2002; Walter and Buchner, 2002). They are grouped into several families based on protein size.

Small Hsps (sHsps) encompass a widespread but diverse class of proteins. These low molecular mass proteins (15–42 kDa) form dynamic oligomeric structures ranging from 9 to 50 subunits and play important role in the cellular defence of prokaryotic and eukaryotic organisms against a variety of internal and external stressors ((Haslbeck, 2002; Narberhaus, 2002). The sHsp family has a similar domain to that of α-crystallin. This domain is perhaps responsible for the highly oligomeric structure of this family (Sun and MacRae, 2005). Multimerization of sHsps is assumed to be crucial for their function as molecular chaperones ensuring correct folding, assembly and transport of newly synthesized polypeptides as well as removing abnormal cellular proteins. Therefore, increased expression of sHsps can extend an organism's tolerance to a variety of environmental insults such as heat, cold, salt, desiccation and oxidants (Feder and Hofmann, 1999; Hayward et al., 2004). sHsps have been suggested to be involved in the inhibition of apoptosis, organisation of the cytoskeleton and establishing the refractive properties of the eye lens in the case of α-crystallin (Haslbeck, 2002). In insects, they are assumed to play a role in the regulation of diapause (Yocum et al., 1998; Rinehart and Denlinger, 2000).

Insect diapause is a dynamic process consisting of several successive phases. Diapause is an endogenously and centrally mediated interruption that routes the developmental program away from direct morphogenesis into an alternative program of arrested development. The start of diapause usually precedes the advent of adverse conditions and the end of diapause need not coincide with the end of adversity (Danks, 1987; Kostal, 2006). Diapause represents an alternative developmental pathway prompted by unique patterns of gene expression. Genes encoding certain stress proteins (Hsp23 and 70) are highly up-regulated during diapause, while others are either unaffected (Hsc70) or are down-regulated (Hsp90) (Denlinger et al., 2001). How Hsps may function in the long-term developmental arrest associated with diapause is unclear. Yocum et al. (1998) suggested that Hsps may persist for long periods during diapause and this is very interesting because extended expression of Hsps can lead to deleterious effects, including retardation and cessation of development (Feder et al., 1992).

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sHsps have been studied in insects, including *Drosophila triaurana*, and *D. melanogaster* (Goto et al., 1998; Goto and Kimura, 2004; Ingolia and Craig, 1982; Frydenberg et al., 2003), the flesh fly, *Sarcophaga crassipalpis* (Yocum et al., 1998), the Indianmeal moth, *Plodia interpunctella* (Shirk et al., 1998), the endoparasitic wasp, *Venturia canescens* (Reineke, 2005), the intertidal copepod *Tigriopus japonicus* (Seo et al., 2006), the leaf beetle, *Gastrophysa atrocyanea* (Atungulu et al., 2006), and the silkworm, *Bombyx mori* (Sakano et al., 2006). The up-regulation of sHsps appears to be common to diapause in species representing diverse insect orders, including Diptera, Lepidoptera, Coleoptera, and Hymenoptera as well as different developmental stages (embryo, larva, pupa, adult) (Rinehart et al., 2007). In contrast, recent studies propose that this is not the case in all insect species studied so far (Goto and Kimura, 2004; Tachibana et al., 2005; Sonoda et al., 2006). Does this link of sHsps expression with diapause occur in other species? What function do the sHsps serve during diapause?

The stalk borer, *Sesamia nonagrioides* (Lefebvre) (Lepidoptera: Noctuidae), is a multivoltine species in the Mediterranean basin; it causes noticeable damage in maize by boring galleries in the stem. This species overwinters as a mature larva and in Greece the first adults appear from early March to early May, in mild and cold areas, respectively (Fantinou et al., 1995). During diapause larvae continue to feed, undergoing supernumerary moults (Fantinou et al., 1998). Photoperiod is the major factor controlling the induction and termination of larval diapause under laboratory conditions, while temperature may also influence the response to daylength (Hilal, 1977; Eizaguirre et al., 1994; Fantinou et al., 1995).

To unravel the potential contribution of *sHsps* transcripts to diapause of *S. nonagrioides*, we isolated two full-length cDNA sequences of *S. nonagrioides* (*SnoHsp19.5* and *SnoHsp20.8*). The expression patterns showed that these genes are rapidly and highly induced after heat stress and suppressed after cold stress. We also observed the presence of the transcript throughout diapause under normal and heat shock conditions. Expression of each gene differs during non-diapause and diapause conditions, indicating that the *SnoHsp19.5* and *SnoHsp20.8* genes may play distinctive roles in the regulation of the diapause process.

2. Materials and methods

2.1. Experimental insects and temperature treatments

The insects were obtained from an established laboratory colony of *S. nonagrioides*, derived from larvae collected in Kopais (latitude 38°14′, Central Greece) in 2006, maintained at 25 ± 1 °C and reared on artificial diet (Tsitsipis, 1984). The colony of non-diapausing insects was reared under long day (LD) conditions (16:8 light:dark) at 25 °C, while diapausing larvae were reared under short day (SD) conditions (10:14 light:dark) at 25 °C. In the following text, the ontogeny that includes diapause will be divided into three main phases: (1) Pre-diapause: larvae grow through the 6th instar. (2) Deep-diapause: during the deep-diapause phase, larvae of *S. nonagrioides* undergo supernumerary moults. (3) Post-diapause: after diapause development, larvae remain under a post-diapause phase, exhibit supernumerary moults and proceed to pupation (Fantinou et al., 1998).

Two different temperature treatments were performed: (i) LD larvae were placed in a polystyrene tube and submerged in a water bath at 40 °C for 15–60 min. (ii) Larvae under LD were exposed to −5 °C, 0 °C, 5 °C, 10 °C, or 17 °C, for 1 h. The cold/heat shocked larvae of *S. nonagrioides* were maintained after treatment at 25 °C at LD. All experimental were conducted in triplicate.

2.2. Molecular techniques

Total RNA was isolated from larvae using TRIzol® reagent (Gibco BRL) according to the supplier's instructions and stored at −80 °C. The concentration of RNA was measured using a spectrophotometer (Spectronic model 21D). DNA was digested with DNase I (Invitrogen) for RT-PCR analyses.

The partial clones for *S. nonagrioides Hsp19.5* and *Hsp20.8* were developed by RT-PCR from an RNA pool derived from non-diapausing larvae (5th instar) reared under LD 16:8 at 25 °C exposed to heat shock (40 °C for 60 min). Total RNA was treated with RNase-free DNAse I (Promega) and 2 μg was used as a template in first-strand cDNA synthesis using Superscript™ II RNase H–Reverse Transcriptase (Invitrogen). Degenerate primers for PCR were designed using consensus sequences from several Lepidoptera (Table 1). The amount of cDNA corresponding to 200 ng of total RNA isolated from larvae was used as the template, together with 200 μM of each dNTP, 20 pmol of each primer, 2 U of DNA polymerase (Expand-High Fidelity, Roche, Mannheim, Germany) in a 50-μl reaction volume. Amplification was achieved in a Peltier thermal cycler (Model PTC-200, M.J. Research, Waltham, MA). After an initial denaturation step at 94 °C for 2 min, 35 cycles were run each with 94 °C for 30 s, 52 °C for 30 s, and 74 °C for 45 s. The PCR products were gel extracted and sequenced. The 3′- and 5′-ends of the cDNA fragment were amplified according to protocols described by Frohman (1990). For 3′-RACE, the first-strand cDNA was primed with the T17XHO primer (Table 1). Based on sequence information of the central cDNA fragment, three *SnoHsp19.5* and two *SnoHsp20.8* specific forward primers were used (Table 1). For 5′-RACE, synthesis of the first-strand cDNA was conducted with the reverse specific primer *Sn*5R1 (*Sn19.5* 5R1 for *SnoHsp19.5* and *Sn20.8* 5R1 for *SnoHsp20.8*) (Table 1). The first-strand cDNA was dA-tailed and amplified with the nested primer *Sn*5R2 (*Sn19.5* 5R2 for *SnoHsp19.5* and *Sn20.8* 5R2 for *SnoHsp20.8*) followed by the primer *Sn*5R3 (*Sn19.5* 5R3 for *SnoHsp19.5* and *Sn20.8* 5R3 for

Table 1
Primer sequences used in this study

Oligonucleotides	Sequence (5′ → 3′)
Degenerate PCR	
Sn20.8 forward	GACTACT(A/T)CAGACC(A/C)TGGAGACA
Sn20.8 reverse	CTCTG(A/G)TCCTTGATCTCCTTGCG
Sn19.5 forward	GACGT(CT)CA(CT)TTC(GT)CGCC(GT)GA
Sn19.5 reverse	GA(AT)ATGAA(GT)CC(AG)TGCTCGTCCT
3′RACE PCR	
Sn20.8 3F 1	CCTGAAGAGATTTCGGTGAAGAC
*Sn*20.8 3F 2	CCAGAAGCCTTAAAGGGAGAGAG
Sn19.5 3F 1	GTTTCCTTTCCCGGCAGTTTAC
Sn19.5 3F 2	TGTGTACCAGAGTCGGTGGAATC
Sn19.5 3F 3	CTAGGAAGGTGCCTCTTGCTG
T17XHO	GTCGACCTCGAGT$_{17}$
5′RACE PCR	
Sn20.8 5R 1	GTGCTCGTCCTTCTTCTCCTCA
Sn20.8 5R 2	GTCTTCACCGAAATCTCTTCAGG
Sn20.8 5R 3	GAGCCCAGATCTTTAGCAGCAG
Sn19.5 5R 1	CTGAACAGCAAGAGGCACCTTC
Sn19.5 5R 2	CAGCTTAGATTCCACCGACTCTG
Sn19.5 5R 3	ACTCTGGTACACAGCCTTCTGG
Sn19.5 5R 4	GTAAACTGCCGGGAAAGGAAAC
Semiquantitative RT-PCR	
Sn20.8 RT-For	GGCTACATCAGTCGGCAGTTC
Sn20.8 RT-Rev	CTCTCTCCCTTTAAGGCTTCTG
Sn19.5 RT-For	ACCAGAGTCGGTGGAATCTAAG
Sn19.5 RT-Rev	GCACTAGATCACATCGCTTCAC
*Sn*RT-TubF	GAGCAGTTCACCGCTATGTTC
*Sn*RT-TubR	GGTGTGAGTGCTTTAGTTGTCC