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Original article

Ultraspiracle of the stingless bees *Melipona scutellaris* and *Scaptotrigona depilis*: cDNA sequence and expression profiles during pupal development*

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Abstract – Reproduction and development in insects and caste differentiation in bees are mainly governed by ecdysteroids and juvenile hormone. We characterized the *ultraspiracle* (nuclear hormone receptor) cDNA of the highly social bees *Melipona scutellaris* and *Scaptotrigona depilis*. The predicted Usp proteins (with 427aa) show a greater sequence similarity to its orthologs from the "ApLoTe" group than to the dipteran-lepidopteran group, suggesting that Usp proteins included in the bee group might share functional characteristics. The expression profiles in fat bodies (FB) and brains turned out not to be very similar between the species. In *M. scutellaris* FB and brain and in *S. depilis* brain *usp* expression anti-parallels pupal ecdysteroid titers, thus suggesting a repression of *usp* expression by this hormone.

ultraspiracle / juvenile hormone / ecdysone / vitellogenin / fat body / brain / Apidae

1. INTRODUCTION

Insect reproduction and development are mainly governed by the lipophilic hormones ecdysteroids and juvenile hormone (JH). In some highly eusocial bees, JH is also responsible for the developmental processes leading to caste differentiation (Velthuis and Velthuis-Kluppell, 1975; Barchuk et al., 2007) and for major behavioral switches occurring in adult workers (Robinson and Vargo, 1997). Ecdysteroids bind nuclear receptor proteins (belonging to the nuclear hormone receptors superfamily) inside the cell which, in turn, results in the activation or the repression of target genes (Koelle et al., 1991). Ultraspiracle (Usp), one of the proteins that constitutes the ecdysone receptor complex together with the ecdysone receptor protein (EcR; Oro et al., 1990; Yao et al., 1993), has ligand-dependent activity in *Drosophila melanogaster* (Jones et al., 2006) and has been suggested to have it in *Apis mellifera* (Barchuk et al., 2004). By heterodimerizing with EcR and HR38 (an orphan nuclear receptor; Sutherland et al., 1995) and by homodimerizing and binding JH (Jones et al., 2006), Usp becomes a pleiotropic regulator of insect development, mediating the action of both main hormones in insects, ecdysteroids and JH. For a review on nuclear hormone receptors in the honeybee *A. mellifera*, see the recent work of Velarde et al. (2006).

Melipona scutellaris Latreille and *Scaptotrigona depilis* Moure are stingless honeybees which, like stinging honeybees, possesses

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high levels of social organization and whose caste differentiation processes are governed by JH and ecdysteroids (see Engels and Imperatriz-Fonseca, 1990; Hartfelder et al., 2006). One of the main phenotypic manifestations of caste differentiation in bees that underlies the reproductive division of labor among females is the production of vitellogenin (vg), the main protein of insect eggs (see Engels et al., 1990). The expression of the *vg* gene has been extensively studied in *A. mellifera* (Engels et al., 1990; Barchuk et al., 2002; Piulachs et al., 2003; Guidugli et al., 2005a, b). The onset of *vg* expression during pupal development in this species occurs in the context of an increasing JH titer and decreasing levels of ecdysteroids (Barchuk et al., 2002; Piulachs et al., 2003). Thus, Usp protein may be mediating the regulation of *vg* expression via its participation in the ecdysone receptor complex or acting as JH receptor. In stingless bees, the expression of the *vg* gene associated with workers' egg-laying capacity has been studied in *S. postica depilis* (*S. depilis*) and *M. scutellaris* (Dallacqua et al., 2006; Hartfelder et al., 2006; Hartfelden, personal communication). The authors showed that in both species (and in *Frieseomellita varia*) *vg* is expressed during the entire pupal stage and adult life. Juvenile hormone and ecdysteroid titers during post-embryonic development have also been determined for these bees, specifically *S. depilis* and *M. quadrifasciata* (Hartfelder and Rembold, 1991; Pinto et al., 2002).

Another phenotypic manifestation of caste differentiation governed by hormones and their nuclear receptors in bees is related to the degree of brain development. The differential nervous system development in workers favors learning and memory-related skills that bees use for navigation, foraging, kin recognition and other activities. In insects, the main nervous system organ in charge of these functions is the mushroom body (MB), an integrative part of the brain (Fahrbach, 2006). The differential growth in MB between queens and workers includes differential proliferation and fasciculation. The high titers of ecdysteroids during larval development in *A. mellifera* queens (Hartfelder and Engels, 1998) likely impair a pronounced MB development, since the mitotic activity of MB cells has been shown to be inhibited by 20E (Ganeshina et al., 2000; Malun et al., 2003).

Multiple alignment analyses have shown that the ligand-binding domain of *A. mellifera* Usp is more similar to that of vertebrates RXR (which binds 9-*cis* retinoic acid; Mangelsdorf et al., 1992) than to those of other insects (diptera and lepidoptera), suggesting the existence of a not yet identified ligand for this nuclear receptor in honeybees (Barchuk et al., 2004). Thus, the existence of a ligand that may induce the activity of a transcription factor can be suggested by phylogenetic studies (see Escriva et al., 2004) and may eventually be demonstrated by biochemical and physiological studies.

Here we characterized the *ultraspiracle* cDNA of *M. scutellaris* and *S. depilis,* which are members of the Apidae family and bees being used in comparative and evolutionary studies of insect polyphenism (Hartfelder, 1987; Hartfelder and Rembold, 1991; Hartfelder and Emlen, 2005; Dallacqua et al., 2006; Vieira et al., 2006; Hartfelder et al., 2006; Cruz-Landim et al., 2006). Our aim was to obtain information about its amino acid sequence, which would allow us to infer information about the eventual existence of ligand-dependent activity of this nuclear hormone receptor in these bees, thus facilitating evolutionary studies. In addition, and with a view to shed light on the molecular mechanisms controlling *vg* expression during pupal stages and the brain development of these bees, we determined the expression profile of *usp* gene in fat bodies and brains during worker pupal development.

2. MATERIALS AND METHODS

2.1. Bees

Pupae were collected from stingless bee colonies maintained at the experimental apiary of the University of São Paulo at Ribeirão Preto, Brazil. When necessary, bees were maintained in an incubator at 28 °C and 80% relative humidity, the same conditions found in the nest. At least 3 groups consisting of 3–7 individuals (for a total of 9–21 bees) were used in each experiment. Fat bodies (where

vg synthesis takes place) and brains were extracted in dissecting dishes on dry ice, suspended in TRIzol reagent (Invitrogen) and frozen at –80 °C until RNA isolation.

2.2. Cloning and sequencing the *ultraspiracle* cDNAs and bioinformatics

Total RNA was extracted from fat bodies and brains using a TRIzol (Invitrogen) protocol or alternatively the GenElute Mammalian Total RNA kit (Cat. RTN70, Sigma) and then incubated in the presence of DNAse I (Promega) for 30 min at 37 °C to eliminate contaminating DNA.

The cloning of Usp cDNAs was performed on PCR amplified nucleic acids obtained using primers based on the sequence of Usp cDNA from *A. mellifera* workers (Barchuk et al., 2004). Database searches were carried out at the National Center for Biotechnology Information using the BLAST server with orthologs aligned by ClustalW. For the molecular phylogeny analysis of Usp, the ClustalW results were converted into Mega 3.1 format (Kumar et al., 2001). Tree construction was performed using the Maximum Parsimony procedure and *Schistosoma mansoni* Usp as out-group.

2.3. Semi-quantitative assay by RT-PCR

First-strand cDNA was synthesized as in Nascimento et al. (2004). Forward N_Usp-1 (5'-TCAAGAGGACCGTACGCAAG-3') and reverse N_Usp-2 (5'-AACAGGTACTCCGGG CAGTT-3') primers were utilized in PCR reactions for *usp* expression quantification. The thermal cycling program was adjusted to the specific primer requirements. For normalization we performed RT-PCR on β-*actin* gene using Act forward (5' TGCCAACACTGTCCTTTCTG 3') and Act reverse (5' AGAATTGACCCACCAATCCA 3') primers based on *A. mellifera* β-*actin* gene (GenBank accession number AB023025). The amplification products, an ~ 800 bp sequence for Usp and ~ 200 bp sequence for β-actin, were run in 1% agarose gels with ethidium bromide. Band intensities were estimated using Tnimage Image Analysis 3.3.7a, and *usp* transcript levels were normalized against the respective levels of β-*actin*, whose expression varies only slightly during development.

3. RESULTS

3.1. *Ultraspiracle* cDNAs and their predicted translation products

The sequenced Usp cDNAs from *M. scutellaris* and *S. depilis* workers have 1284 and 1665 bp, respectively, including an open reading frame coding for 427 amino acids. Both sequences have been deposited in GenBank under the accession numbers AY840093 (*M. scutellaris*) and DQ190542 (*S. depilis*). The predicted translation products have an estimated pI value of 8.80 and 8.87 (respectively) and a molecular weight of approximately 48 kDa (pI/Mw tool, http://www.expasy.org/tools/pi_tools.html).

The sequenced Usp cDNAs from *M. scutellaris* and *S. depilis* encode very similar polypeptides. The high level of similarity with those of other animal species (see Figs. 1, 2) and the modular organization of the translated proteins indicate that the cDNAs code for a member of the nuclear hormone receptor superfamily, specifically for the ultraspiracle protein, an ortholog of the vertebrate RXR (Henrich and Brown, 1995; Riddiford et al., 2000; King-Jones and Thummel, 2005). The stingless bees Usp, thus, include the 4 main domains of this kind of transcription factor (Fig. 1): the N-terminal A/B, C domain (DBD, DNA-binding domain), D (hinge domain), and the carboxiterminal E/F domains (LBD, ligand-binding domain).

Usp of the analyzed bees also possesses the conserved sequences localized in specific regions of the main domains. The consensus sequences that confer DNA binding specificity (EGCKG, P-box) and dimerisation capacity (REEKS, D-box) can be identified in the DBD (italicized and bold in Fig. 1). Ultraspiracle of different species normally have a consensus sequence of 10 amino acids in the D (hinge) domain (REAVQEERQR). In the studied bees, we found the sequence REAVHEERQR in *M. scutellaris* and RGAVQEERQR in *S. depilis*. Thus, the differences in Usp sequences between these two bees are concentrated in D domain: the base in position 533 is a "g" in *S. depilis,* resulting in the non-polar amino acid glycine (G) in place of the negatively charged

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ATGATGAAGAAGGAGAAGCCGATGATGTCGGTGACCGCTATCATCCAAGGGACCCAGGCT - 60
M  M  K  K  E  K  P  M  M  S  V  T  A  I  I  Q  G  T  Q  A
CAGCATTGGTCCCGTGGGAACACTTGGCTGAGTTTGGACAACAGTAACATGTCCATGTCC - 120
Q  H  W  S  R  G  N  T  W  L  S  L  D  N  S  N  M  S  M  S
TCGGTGGGCCCGCAAAGTCCGCTGGACATGAAGCCTGACACAGCTAGCCTCATCAATCCA - 180
S  V  G  P  Q  S  P  L  D  M  K  P  D  T  A  S  L  I  N  P
GGAAACTTCAGTCCTTCCGGTCCCAATAGTCCTGGATCCTTCACCGCTGGTTGTCACAGC - 240
G  N  F  S  P  S  G  P  N  S  P  G  S  F  T  A  G  C  H  S
AACCTTCTGAGTACGTCGCCGAGTGGACAGAACAAAGCGGTCGCGCCCTATCCGCCGAAC - 300
N  L  L  S  T  S  P  S  G  Q  N  K  A  V  A  P  Y  P  P  N
CACCCTCTATCTGGCAGCAAGCACCTCTGCTCGATCTGCGGTGACCGTGCCAGTGGCAAG - 360
H  P  L  S  G  S  K  H  L  C  S  I  C  G  D  R  A  S  G  K
CATTACGGTGTCTATAGCTGCGAGGGTTGCAAGGGTTTCTTCAAGAGGACCGTACGCAAG - 420
H  Y  G  V  Y  S  C  E  G  C  K  G  F  F  K  R  T  V  R  K
GACCTGTCGTACGCGTGCCGCGAGGAGAAGTCGTGCATCATCGACAAACGACAGAGAAAC - 480
D  L  S  Y  A  C  R  E  E  K  S  C  I  I  D  K  R  Q  R  N
CGATGCCAGTATTGCAGATACCAGAAATGCCTGGCGATGGGCATGAAGAGAGAAAGCGGTC - 540
R  C  Q  Y  C  R  Y  Q  K  C  L  A  M  G  M  K  R  E  A  V
CATGAAGAACGCCAGCGTACCAAGGAGAGGGATCAGAGCGAGGTGGAGAGCACCAGTAGC - 600
H  E  E  R  Q  R  T  K  E  R  D  Q  S  E  V  E  S  T  S  S
TTGCACTCGGACATGCCGATCGAGCGCATCCTCGAAGCCGAGAAACGAGTCGAATGCAAA - 660
L  H  S  D  M  P  I  E  R  I  L  E  A  E  K  R  V  E  C  K
ATGGAACAGCAAGGGAATTACGAGAATGCAGTGTCGCACATTTGCAACGCCACGAACAAA - 720
M  E  Q  Q  G  N  Y  E  N  A  V  S  H  I  C  N  A  T  N  K
CAGCTGTTCCAGCTGGTAGCATGGGCGAAACACATCCCGCATTTTACGTCGTTGCCACTG - 780
Q  L  F  Q  L  V  A  W  A  K  H  I  P  H  F  T  S  L  P  L
GAGGATCAGGTACTTCTGCTCAGGGCCGGTTGGAACGAGTTGCTGATAGCCTCCTTTTCC - 840
E  D  Q  V  L  L  L  R  A  G  W  N  E  L  L  I  A  S  F  S
CACCGTTCCATCGACGTGAAGGACGGTATCGTGCTGGCCACGGGGATCACCGTGCACCGG - 900
H  R  S  I  D  V  K  D  G  I  V  L  A  T  G  I  T  V  H  R
AACTCGGCGCAGCAGGCTGGCGTCGGCACGATATTCGACCGAGTGCTTTCCGAGCTTGTG - 960
N  S  A  Q  Q  A  G  V  G  T  I  F  D  R  V  L  S  E  L  V
TCGAAAATGCGCGAAATGAAGATGGACAGGACGGAGCTTGGCTGTTTGAGATCTATAATA - 1020
S  K  M  R  E  M  K  M  D  R  T  E  L  G  C  L  R  S  I  I
CTCTTCAATCCCGAGGTCCGAGGACTGAAATCCATCCAGGAAGTGACCCTGCTCCGCGAG - 1080
L  F  N  P  E  V  R  G  L  K  S  I  Q  E  V  T  L  L  R  E
AAGATCTACGCAGCTCTGGAGGGTTATTGCCGCGTAGCCTGGCCGGACGACGCTGGAAGA - 1140
K  I  Y  A  A  L  E  G  Y  C  R  V  A  W  P  D  D  A  G  R
TTCGCGAAGTTGTTGTTACGCTTGCCGGCCATCCGATCGATAGGACTAAAGTGCCTCGAG - 1200
F  A  K  L  L  L  R  L  P  A  I  R  S  I  G  L  K  C  L  E
TACCTGTTCTTTTTCAAAATGATCGGGGACGTGCCGATCGACGACTTCCTCGTCGAGATG - 1260
Y  L  F  F  F  K  M  I  G  D  V  P  I  D  D  F  L  V  E  M
CTAGAGTCACGATCGGACCCTTAG - 1284
L  E  S  R  S  D  P  *
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Figure 1. Nucleotide and conceptual amino acid sequences of *usp* cDNA from the stingless bees *M. scutellaris* and *S. depilis*. The predicted polypeptide shown by a one-letter code below the respective nucleotide sequence corresponds to *M. scutellaris usp*. The differences respect to the sequences of *S. depilis* are as follows: the nucleotide with an "A" in position 533 is a "G" resulting in a glycine (G) in place of the glutamate (E); and the "T" in position 543 is a "G", resulting in a glutamine (Q) in place of the histidine (H) (the respective codons are underlined). Amino acids belonging to the conserved DBD (C domain) are shaded in light grey and those belonging to E/F domain are shaded in dark grey. Conserved amino acid sequences in C, D and E/F domains are bold and italicized.

glutamic acid (E); and in position 543 there is a "g", resulting in the polar amino acid glutamine (Q) in place of the positively charged histidine (H).

The molecular phylogeny analyses of DBDs resulted in phylogenetic relationships that generally reflect the current phylogeny, showing vertebrate and invertebrate lineages split with high bootstrap values (not shown). The dendrogram based on entire proteins (Usp/RXR) analysis, on the other hand, shows bees Usp together with that of *Locusta*,

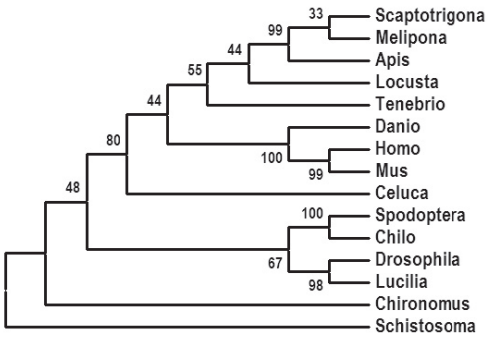

Figure 2. Dendrogram based on entire proteins analysis using Maximum Parsimony procedure, illustrating the evolutionary relationships among USPs/RXRs. *A. mellifera* = AY273778; *M. scutellaris* = AY840093; *S. depilis* = DQ190542; *L. migratoria* = AF136372; *T. molitor* = AJ251542; *C. pugilator* = AF032983; *D. rerio* = AAC59720; *M. musculus* = XM_123763; *H. sapiens* = NM_002957; *C. tentans* = AF045891; *D. melanogaster* = X53417; *L. cuprina* = AY007213; *C. suppressalis* = AF016368; *S. frugiperda* = U06073; *S. mansoni* = AF158102.

Tenebrio and *Celuca* and the RXR of vertebrates (ApLoTe group, Barchuk et al., 2004) clearly separated from the rest of insects Usp, like dipterans and lepidopterans (Fig. 2).

3.2. Expression profile of *M. scutellaris* and *S. depilis* usp in fat bodies and brains

We determined the expression profile of *usp* in fat bodies and brains during pupal development (Fig. 3). There was found little similarity between the species, except for the fact that the highest levels of *usp* expression in both organs and species are concentrated in the earliest stage of pupal development (Pw; see Tab. I), generally diminishing in the last stages. Interestingly, in *M. scutellaris* FB and brain and in *S. depilis* brain *usp* expression antiparallels pupal ecdysteroid titers found in *M. quadrifasciata* and *S. depilis* hemolymph (see Hartfelder and Rembold, 1991; Pinto et al., 2002; Hartfelder et al., 2006).

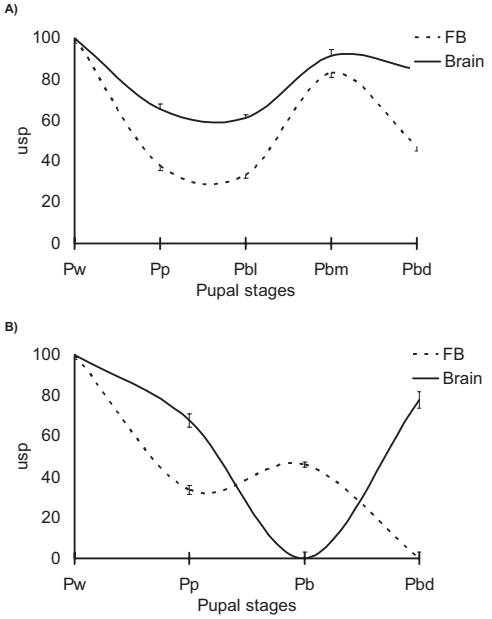

Figure 3. *Usp* expression profiles in fat bodies (FB) and brains during pupal development of *M. scutellaris* (A) and *S. depilis* (B). The ordinate represents densitometric units and each value is an average of three separate experiments. SEMs are indicated by vertical bars. See Table I for the complete name of developmental stages.

4. DISCUSSION

4.1. *Ultraspiracle* from highly eusocial bees might share functional characteristics

We have sequenced the Usp cDNAs from the stingless honeybees *M. scutellaris* and *S. depilis*. Their predicted translation products have an estimated molecular weight of 48 kDa. This value is identical to that estimated for Usp from *A. mellifera* (Barchuk et al., 2004) and similar to those from *Aedes aegypti* (51 and 54 kDa; Kapitskaya et al., 1996), *Lucilia cuprina* (51 kDa; Hannan and Hill, 2001), *Chilo supppressalis* (47 kDa; Minakuchi et al., 2003) and the large isoform of *Blattella germanica* Usp (48.7 kDa; Maestro et al., 2005). The Usp cDNAs from these two bees encode very similar polypeptides. The eventual meaning of the amino acid differences we found in the hinge

Table I. External morphological characters used to stage developing pupae of *M. scutellaris* and *S. depilis* workers (see Dallacqua et al., 2006).

Pupal stage	Eye pigmentation	Body pigmentation
(Larva)		
Pw	white	unpigmented
Pp	pink	unpigmented
Pb	brown	unpigmented
Pbm	dark brown	pigmented appendages; light-pigmented thorax and abdomen
Pbd	dark brown	dark-pigmented appendages; light-pigmented thorax and abdomen
(Adult)		

domain could be assessed by testing Usp behavior using recombinant molecules for the hinge domain or even the original proteins in cell culture assays. Nevertheless, since the D domain does not seem to have critical functions, these differences might not confer functional divergences in Usp of these two bee species (Henrich and Brown, 1995; Riddiford et al., 2000).

The molecular phylogeny analyses of the entire proteins (Usp/RXR), shows bees Usp together with that of *Locusta, Tenebrio* and *Celuca* and the RXR of vertebrates (ApLoTe group, Barchuk et al., 2004) separated from the rest of insects Usp. These results suggest a major contribution of the LBD on this clustering and that the Usp proteins included in the bee group might share functional characteristics.

Since the vertebrate RXR (Usp ortholog) has ligand-binding capacity, binding the 9-*cis* retinoic acid (Mangelsdorf et al., 1992), bees Usp might also have ligand-binding activity, as suggested by the sequence similarity among Usp from the ApLoTe group (see Barchuk et al., 2004; Fig. 2). Moreover, more than one ligand, with different affinities can exist within bees and other insect members (Barchuk et al., 2004). These ligands include metabolites of JH-III, such as farnesol, farnesoic acid, methyl farnesoate and juvenile hormone acid. Nonetheless, since the phylogenetic position of a receptor is clearly not correlated with its liganded/orphan status (Escriva et al., 2004), the confirmed existence of ligand-binding activity of Usp within the insect members of ApLoTe group awaits experimental evidence.

4.2. High ecdysteroid titers seem to repress *usp* expression in stingless honeybees

To learn more of the existence of an occasional hormonal modulation of *usp* gene expression and the relative participation of Usp protein in the *vitellogenin* gene expression regulation and brain development in *M. scutellaris* and *S. depilis*, we determined the expression profile of *usp* in fat bodies (FB, where vg synthesis takes place) and brains during pupal development. The obtained profiles turned out not to be very similar between the species. In *M. scutellaris* FB and brain and in *S. depilis* brain *usp* expression anti-parallels pupal ecdysteroid titers, thus suggesting a repression of *usp* expression by this hormone. This is the case, for example, for *Choristoneura fumiferana*, where *Cfusp* seems to be down-regulated by high titers of 20E (Perera et al., 1998). Moreover, *vg* gene expression during pupal stages seems to be repressed by ecdysteroids in these bees, as has been shown in *A. mellifera* (Barchuk et al., 2002), whose ecdysteroid titers during this period are similar to those of *M. quadrifasciata* and *S. depilis* (Hartfelder and Engels, 1998; Hartfelder et al., 2006). The observed profiles of ecdysteroids and *usp* expression suggest a possible role of Usp protein in *vg* expression regulation in these bees, at least in *M. scutellaris*.

The occasional repression of *usp* by ecdysteroids is interesting since Usp is considered to

be an ecdysone receptor complex constituent, and so we would expect that its gene expression would be induced by these hormones (see Auzoux-Bordenave et al., 2005; Tata, 2006). These results suggest that the *usp* gene is repressed by the mediating ecdysone receptor complex (EcR/Usp+20E) or that there is more than one isoform of Usp in these bees, where one of them is repressed by ecdysteroids and the other induced. This is true for *Manduca sexta*, where two Usp isoforms were found, Usp-1 and Usp-2, the first repressed by ecdysteroids and the second induced (Hiruma et al., 1999). In bees, it has been demonstrated that *A. mellifera* possesses two *usp* transcripts differentially expressed in the bee's body, one with ~ 4 kb and the other with ~ 5 kb, and that *usp* gene expression is induced by JH, while ecdysteroids do not seem to have any effect on the expression of this gene (Barchuk et al., 2004). In contrast, apoptosis processes during pupal brain development seem to be triggered by high ecdysteroid titers around the first stages of this developmental period (Ganeshina et al., 2000; Malun et al., 2003). The observed absence of the correspondent *usp* transcript during this period in *M. scutellaris* and *S. depilis* (see Fig. 3; similar results were obtained in *A. mellifera*, ARB and ZLPS, unpubl. data) would mean its negative participation in this process or, again, its participation through an unknown isoform.

In *S. depilis, usp* expression levels in brains are high during the first two pupal stages and fall abruptly in the third stage, thus differing from the profile found in *M. scutellaris* (Fig. 3). Comparative morphological studies of brain development including estimates of relative consequences of programmed cell death in both bee species are necessary to test possible differential brain development. The confirmation of this idea would have physiological implications, explaining for example, some aspects of the known differences in cell provisioning and feeding behaviors between *Melipona* and *Scaptotrigona* (see Michener, 1974). Additional and more accurate analyses of gene expression levels might help in determining if these differences are meaningful.

The high levels of *usp* expression we found in the last pupal stage brains seem to represent a response to an increase of JH titers which might occur during this period (see Hartfelder et al., 2006), as seen in *A. mellifera* (ARB and ZLPS, unpubl. data; Hartfelder and Engels, 1998). In this bee species, *usp* gene expression is rapid and transiently up-regulated by JH, more likely after the formation of an active Usp-JH receptor complex (Barchuk et al., 2004, 2007; Guidugli et al., 2005a, b). Thus, the relationship between hormones and their nuclear receptors with the *vg* gene expression regulation and brain development in bees (Dallacqua et al., 2006; Hartfelder et al., 2006; Barchuk et al., 2007) are challenging processes that need further investigation.

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Le gène *ultraspiracle* des abeilles sans aiguillon *Melipona scutellaris* et *Scaptotrigona depilis* : séquence de l'ADNc et profils d'expression au cours du développement nymphal.

***ultraspiracle* / hormone juvénile / vitellogénine / corps gras / cerveau / abeille sans aiguillon**

Zusammenfassung – Das *Ultraspiracle* Gen der Stachellosen Bienen *Melipona scutellaris* und *Scaptotrigona depilis*: cDNA Sequenz und Expressionsmuster während der Puppenphase. Die Entwicklung und Reproduktion von Insekten unterliegt im wesentlichen der Steuerung durch lipophile Hormone, insbesondere Ecdysteroide und Juvenilhormon (JH). Bei einigen der hocheusozialen Bienen spielt JH ausserdem eine wichtige Rolle in der Kastenentwicklung und in Verhaltensänderungen, die die Adultphase von Arbeiterinnen kennzeichnen. Durch die Bindung an die Rezeptorproteine Ultraspiracle (Usp) und Ecdysonrezeptor (EcR) im Zellkern regulieren Ecdysteroide die

Expression von Zielgenen. Usp hat die Möglichkeit sowohl als Heterodimer eine Bindung an EcR oder HR38 (einem anderen nukleären Rezeptorprotein) einzugehen, als auch ein Homodimer mit einem anderen Usp-Molekül zu bilden und dann JH zu binden. Damit kann Usp zu einem pleiotropen Faktor werden, der die Insektenentwicklung steuert, indem es die Wirkung beider Insektenhormone, der Ecdysteroide und von JH, in entscheidender Weise beeinflusst.

Die vorliegende Arbeit präsentiert Daten zur Charakterisierung der *ultraspiracle* Komplementär-DNA (cDNA) von zwei Mitgliedern der Familie Apidae, der Stachellosen Bienen *Melipona scutellaris* und *Scaptotrigona depilis*. Unser Ziel war es, Informationen über die entsprechenden Aminosäurensequenzen zu erhalten, die dann in evolutive Analysen Eingang finden können. Insbesondere im Hinblick auf molekulare Mechanismen der Steuerung der Produktion des Dotterproteins Vitellogenin untersuchten wir ausserdem die Expressionsmuster des *usp* Gens im pupalen Fettkörper und in Gehirnen von Arbeiterinnen dieser beiden Arten. Puppenstadien dieser Stachellosen Bienen wurden aus Kolonien entnommen, die im Meliponarium der Universidade de São Paulo, Ribeirão Preto, Brasilien, gehalten wurden. Die Klonierung und Sequenzierung der *usp* cDNAs erfolgte durch PCR Amplifikation mittels spezifischer Primer für die *Apis mellifera usp* cDNA Sequenz. Für die Bestimmung der *usp* Expressionsmuster benutzten wir einen semiquantitativen RT-PCR Ansatz.

Die *usp* cDNA Sequenzen für *M. scutellaris* und *S. depilis* umfassen 1284, bzw. 1665 Basenpaare, die für sehr ähnliche Polypeptide von 427 Aminosäuren kodieren. Diese Werte und Sequenzen sind identisch mit denen der Honigbiene und sehr ähnlich mit denen der Stechmücke *Aedes aegypti*, der Fliege *Lucilia cuprina*, des Reis-Stammbohrers *Chilo suppressalis* und auch mit der grossen Usp-Isoform der Schabe *Blattella germanica*.

Die *usp* Expressionsmuster zeigten erstaunlich wenig Gemeinsamkeiten zwischen den beiden Bienenarten, abgesehen von der Tatsache, dass die Transkriptionsraten im Gehirn und Fettkörper jeweils zu Beginn der Pupalentwicklung (Pw; siehe Tab. I und Abb. 3) am höchsten waren und zum Ende der Puppenphase abnahmen. Interessanterweise zeigten die Expressionsmuster im Fettkörper und in Gehirnen von *M. scutellaris* und in Gehirnen von *S. depilis* einen entgegengesetzten zu den Ecdysteroid-Titern der Hämolymphe dieser beiden Arten, was auf eine mögliche negative Kontrolle der *usp* Expression durch diese Hormonklasse hinweist.

Bei *S. depilis* fanden wir in Gehirnen der frühen Pupenstadien eine hohe *usp* Transkriptionsrate, die dann in dem mittleren Stadium stark abfiel und damit stark von den bei *M. scutellaris* ermittelten Werten abwich. (Abb. 3). Die hohen Werte der *usp* Expression in Gehirnen der späten Puppenstadien weisen auf eine Steuerung durch JH hin (Hartfelder et al., 2006), dessen Titer bei der Honigbiene *A. mellifera* in diesen Stadien langsam ansteigt.

***ultraspiracle* / Juvenilhormon / Ecdyson / Vitellogenin / Fettkörper / Gehirn**

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